Open access • Posted Content • DOI:10.1101/2021.07.18.21260732

Immunogenicity of mRNA-1273, BNT162b2 and Ad26.COV2.S COVID-19 vaccines

Vivek Naranbhai, Wilfredo F. Garcia-Beltran, Christhian Berrios Mairena, Julia Thierauf ...+21 more authors

Institutions: Harvard University, University of New South Wales

Published on: 13 Oct 2021 - medRxiv (Cold Spring Harbor Laboratory Press)

Topics: Immunogenicity

Immunogenicity of mRNA-1273, BNT162b2 and Ad26.COV2.S COVID-19 vaccines

Vivek Naranbhai MBChB, PhD, DPhil[1,2,3], Wilfredo F. Garcia-Beltran MD, PhD[1], Cristhian Berrios Mairena MD[1], Julia C. Thierauf MD, PhD [1], Christina C. Chang MBBS, PhD[3,4,5], Grace Kirkpatrick BS[1], Maristela L. Onozato MD, PhD[1], Ju Cheng PhD[1], Kerri J. St. Denis BA[1], Evan C. Lam BS[1], Clarety Kaseke BS[1], Rhoda Tano-Menka BS[1], Diane Yang PhD[1], Maia Pavlovic BS[1], Wendy Yang BS[1], Alexander Kui, BS[1], Tyler E. Miller, MD, PhD[1], Michael G. Astudillo, MD[1], Jennifer E. Cahill PhD, RN[1], Anand S. Dighe, MD, PhD[1], David J. Gregory PhD[1], Mark C. Poznansky, MD,PhD,[1] Gaurav D. Gaiha, MD, PhD[1], Alejandro B. Balazs PhD[1], A. John Iafrate MD, PhD[1]*

[1]Massachusetts General Hospital, Boston USA

[2]Dana-Farber Cancer Institute, Boston, USA

[3]Center for the AIDS Programme of Research in South Africa, Durban, South Africa

[4]University of New South Wales, Sydney, Australia

[5]Monash University, Melbourne, Australia

*Corresponding author: aiafrate@partners.org

Manuscript word count: 1344 words

Abstract [242/250 words]

Background: Understanding variation in immunogenicity may help rationalize use of existing SARS-CoV-2 vaccines.

Methods: We compared immune responses in ambulatory adults vaccinated with mRNA-1273 , BNT-162b2 or Ad26.COV2.S in Massachusetts, USA between February and May 2021. Control groups were pre-pandemic controls (n=1220) and individuals without (n=112) or with prior SARS-CoV-2 infection (n=130) sampled in mid-2020. We measured total anti-spike IgG/M/A antibodies (Roche Elecsys Anti-SARS-COV-2 S assay), anti-receptor-binding-domain (RBD) antibodies; neutralization of SARS-CoV-2 pseudovirus; and T-cell responses.

Findings In individuals with prior infection, all vaccines were associated with higher antibody concentrations and neutralization than those in convalescent individuals, even after a single dose. In individuals without prior infection, a single dose of either mRNA vaccine yielded comparable concentrations and neutralization to convalescent unvaccinated individuals, and Ad26.COV2.S yielded lower antibody concentrations and neutralization titers. The second dose of either mRNA vaccine boosted responses. At a median of 24 days after vaccination, two of 21 (9.5%) Ad26.COV2.S recipients had a neutralization titer higher than pre-pandemic controls; repeat sampling at a median 66 days after vaccination found most (11/15 (73%) remained negative. Antibody concentrations and neutralization titers increased similarly after the first dose of either vaccine, and even further in recipients of a second dose of vaccine. T-cell responses were higher in mRNA1273 and BNT162b2 than Ad26.COV2.S recipients.

Interpretation

SARS-CoV-2 vaccines vary significantly in immunogenicity in individuals without prior infection. If confirmed in effectiveness studies, public health policy may need to be tailored to each vaccine, or even individual responses.

Main Text [1344 words]

Prophylactic vaccines against SARS-CoV-2 are being deployed globally to combat the COVID-19 pandemic. There are few data regarding the comparative immunogenicity of these vaccines, except for indirect inferences from publicly available trial data from manufacturers[1–6]. Immunogenicity measures of different vaccines may be important to quantitate as we seek to understand variation in vaccine effectiveness between individuals.

We characterized the immunogenicity of mRNA-1273 (Moderna), BNT162b2 (Pfizer-BioNTech) and Ad26.COV2.S (Johnson & Johnson (Janssen)) in ambulatory adults enrolled in an ongoing community study in Chelsea, Massachusetts and a vaccine biobanking effort in Boston, Massachusetts. In total we include data from 215 participants who had received one (n = 99) or two doses (n = 116) of vaccine ≥7 days prior, 130 unvaccinated participants with asymptomatic or symptomatic prior infection confirmed by positive anti-nucleocapsid antibody, 112 uninfected (anti-nucleocapsid antibody negative), and 1,220 historical controls sampled before the pandemic[7]. The median age of vaccinated participants was 39 years (IQR 31-55 years) and 120/215 (56%) were female (**Supplementary Table 1**). We compared immune responses according to prior infection, and vaccination type and dose and adjusted for age, sex and duration after vaccination in all analyses.

We assessed total IgG/M/A binding antibody levels against the SARS-CoV-2 spike protein (Roche Elecsys Anti-SARS-CoV-2 S), and found substantial variation in antibody concentrations depending on prior infection, vaccine type and vaccine dose (**Figure 1A**, summarized in a multivariate regression model in **Supplementary Table 2).** Among participants without prior infection, antibody concentrations after a single dose of mRNA-1273 or BNT162b2 were comparable or slightly lower than convalescent individuals (geometric mean concentration in U/mL (GMC) 222 vs. 189, adjusted p=0.4 for mRNA-1273 and 71 vs. 189, adjusted p=0.01 for BNT162b2). In contrast, recipients of Ad26.COV2.S with no prior infection had significantly lower antibody concentrations than convalescent unvaccinated individuals (GMC 6.9 vs 189, adjusted p <0.001). Although participants with no prior infection who received a single dose of either of the three vaccines were sampled at a comparable duration after vaccination (a median of

22-24 days), a single dose of mRNA-1273 or BNT162b2 generated higher titers than Ad26.COV2.S (adjusted $p < 0.001$ for both comparisons, **Supplementary Table 3**). At a median of 24 days post-vaccination, seven of twenty-two (17.3%) Ad26.COV2.S recipients had undetectable antibody levels. We confirmed that all 22 Ad26.COV2.S recipients had no major medical comorbidity and were not on immunosuppressive medications in the prior six months. Receipt of both doses of mRNA-1273 or BNT162b2 were associated with substantially higher antibody concentrations than convalescent individuals (GMC 6486 for mRNA-1273 and GMC 2455 for BNT162b2 vs. 189, adjusted $p<0.001$ for both comparisons). Individuals with prior infection who were vaccinated had ~2 $\log_{10}$ IU/ml higher antibody concentrations than convalescent individuals regardless of vaccine type, even after a single dose and there was a non-significant pattern towards lower concentrations in Ad26.COV2.S recipients. Using an independent validated total IgG/M/A or IgG ELISA, measurement of receptor binding domain (RBD) binding antibodies confirmed the differences above; and IgM and IgA responses were low (**Figure S1**). Further measurement of IgG against RBD-multimers also confirmed these findings (**Figure S2**). IgG responses against equivalent RBD-multimers of less pathogenic, common coronaviruses OCU43 and HKU1 were comparable between groups supporting the specificity of this finding (**Figure S3**).

Correlative analysis of the SARS-CoV-2 vaccine trials demonstrate that neutralizing antibody titer is associated with the degree of protection observed[8,9]. In animal models, experimental transfer of antibodies protects from infection[10,11] and in human randomized controlled-trials, prophylactic administration of neutralizing antibodies reduces incidence of clinical COVID-19 [12,13]. We assessed neutralization of pseudoviruses representing the original Wuhan isolate of SARS-CoV-2, which formed the basis of all three vaccines, in all Ad26.COV2.S recipients and a random subsample of mRNA-1273 and BNT162b2 vaccinees (total 190 vaccinees). Among pre-pandemic and confirmed uninfected individuals, a neutralization titer (pNT50) cutoff of 1:20 identified 1.6% as positive[7]. Using this threshold, 90.1% of unvaccinated convalescent individuals demonstrated neutralization (**Figure 1B**). Following a single dose of mRNA1273 or BNT162b2, neutralization titers were comparable to convalescent individuals (geometric mean titer (GMT) 115 for mRNA1273 and 94 for BNT162b2 vs. 139 for convalescent donors, p=ns for both comparisons **Supplementary Table 4**) and 78% and 86% neutralized virus. Titers were

higher after both doses of mRNA vaccine (GMT 715 for mRNA1273 and 927 for BNT162b2, adjusted $p<0.001$ for both), and serum from all mRNA vaccinees neutralized wild-type SARS-CoV-2 pseudovirus. In contrast, only two of 21 (9.5%) Ad26.COV2.S recipients had a neutralization titer >1:20. Among individuals with prior infection, receipt of one or two doses of either of the three vaccines, generated high neutralization titers.

To evaluate the described increase in anti-SARS-CoV-2 antibodies at later timepoints in Ad26.COV2.S recipients[2,3], we obtained repeat measures in a subset of 15 Ad26.COV2.S recipients without prior infection For this subset the baseline sampling was at a median 23 (range 7-44) days and follow-up at a median 66 (range 25-82) days after vaccination. Anti-spike antibody concentrations increased modestly for all Ad26.COV2.S individuals (**Figure 2A**); however, only four individuals had an increase in neutralization, and 11/15 (73%) remained <1:20 (**Figure 2B**). Next, we pooled initial and repeat measures among individuals without prior infection and modelled the kinetics of responses for all three vaccines. Over the first ~6 weeks following receipt of first dose of vaccination, antibody concentrations increased regardless of vaccine (**Figure 2C**) but the estimated plateau titers were lower amongAd26.COV2.S recipients. Constraining analysis to the period prior to the second mRNA vaccine effect (ie. the first four weeks for BNT162b2 and five weeks for mRNA1273), titers increased for all three vaccines, and the rate of increase was not statistically dissimilar between vaccines (interaction $p=0.85$). In this cohort, 80% of convalescent individuals have a pNT50 of greater than 45.6, corresponding with published estimates of the threshold for ~50% protection[8]. Using this threshold, most recipients of mRNA-1273 and BNT162b2 achieved predicted protective neutralization titers rapidly, before receipt of the second dose of vaccination, and these appear to be sustained for several months (**Figure 2D**). In contrast, most Ad26.COV2.S recipients did not neutralize virus even after 6 weeks. Since previously published studies of Ad26.COV2.S recipients that have described a more robust increase in neutralization utilized the Victoria strain of SARS CoV-2 [2,3] , a strain that has been noted to be more easily neutralized[14], we introduced the Victoria strain-associated S247R mutation into the SARS CoV-2 Wuhan isolate used in this study. Sera from vaccine recipients demonstrated higher neutralization titers against this S247R-containing variant than the original Wuhan strain regardless of vaccine administered (**Figure S4**). Therefore differences

in the described neutralization seen in this study vs. the Ad26.COV2.S trials[2,3] may be partially accounted for by use of the S247R viral variant in prior studies.

To explore differences in cellular immune response to vaccination, we assessed T-cell responses to SARS-CoV-2 spike peptides (wild-type strain) by IFN-γ ELISpot in a subset of uninfected vaccinees who completed their vaccination schedule (**Figure 3**). For reference, unvaccinated participants with prior infection had a mean 170 spot forming units (SFU) per 10^6 PBMC. Spike-specific T-cell responses varied significantly by vaccine (Kruskall-Wallis $p<0.001$). T-cell responses were higher in recipients of mRNA-1273 or BNT-162b2 than Ad26.COV2.S (mean 564 vs. 96 SFU/10^6 PBMC, adjusted p=0.003) and interestingly responses were also higher in mRNA1273 than in BNT-162b2 recipients (mean 564 vs 398 SFU/10^6 PBMC, adjusted p=0.04).

In summary, these data suggest marked differences in the immunogenicity of mRNA-based vaccines (mRNA-1273 and BNT162b2) and Ad26.COV2.S in the first weeks following vaccination in healthy individuals. Differences in the efficacy[1,5,6] and effectiveness during deployment under emergency-use-authorization of mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines may be, at least in part, due to the variable immunogenicity of the vaccines described here. Among individuals with prior infection, vaccination conferred higher antibody concentration and neutralization titers regardless of vaccine type and whether a second dose was given; efficacy and effectiveness studies should be stratified by prior infection status of participants. This supports recent data suggesting a single dose of mRNA vaccine in seropositive convalescent patients elicits comparable antibody titers to seronegative individuals who receive two doses of mRNA vaccine. The sustained lower immunogenicity of the Ad26.COV2.S vaccine by multiple measures used in this study warrants further study about the possible benefit of booster doses.

Acknowledgements

We thank Andrea Nixon and the MGH Core laboratory for assistance in performing assays. We wish to thank Michael Farzan, PhD for providing ACE2-expressing 293T cells and Aaron Schmidt for providing proteins for development of ELISA assays and Atul Bhan, MD and Mandakolathur Murali, MD for useful and insightful discussion. The RBD ELISA assay was

developed by Steve Mullenbrock, Diego Farfan-Arribas, Mark Stump, Nels Pederson, Randy Wetzel and Roberto Polakiewicz of Cell Signaling Technologies, Danvers, MA. We also thank Kellie Burke, Susan Gonzalez, Dominic Iafrate, Chioma Agugoesi, Katie Tarbox, Kirsten Dickens, Pedro Ojeda, JeanCarlos Cruz, Julian Villalba, Michelle Garlin, Daniel Montes, Karla Gonzalez, Hugh Shirley, Flor Amaya, Mimi Graney, Daniel Cortez and Minjee Kim for assisting in participant recruitment. D.J.G., M.C.P., and M.N.P. were supported by the VIC Innovation fund. A.J.I. and this study were supported by the Lambertus Family Foundation. G.D.G. is supported by a Burroughs Wellcome Career Award in Medical Sciences. A.B.B. was supported by the National Institutes for Drug Abuse (NIDA) Avenir New Innovator Award DP2DA040254, the MGH Transformative Scholars Program as well as funding from the Charles H. Hood Foundation.

References

1. Sadoff J, Gray G, Vandebosch A, et al. Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against Covid-19. *The New England journal of medicine*. Published online April 21, 2021. doi:10.1056/NEJMoa2101544
2. Stephenson KE, le Gars M, Sadoff J, et al. Immunogenicity of the Ad26.COV2.S Vaccine for COVID-19. *JAMA - Journal of the American Medical Association*. 2021;325(15):1535-1544. doi:10.1001/jama.2021.3645
3. Sadoff J, le Gars M, Shukarev G, et al. Interim Results of a Phase 1–2a Trial of Ad26.COV2.S Covid-19 Vaccine. *New England Journal of Medicine*. Published online May 13, 2021. doi:10.1056/nejmoa2034201
4. Anderson EJ, Rouphael NG, Widge AT, et al. Safety and Immunogenicity of SARS-CoV-2 mRNA-1273 Vaccine in Older Adults. *New England Journal of Medicine*. 2020;383(25):2427-2438. doi:10.1056/nejmoa2028436
5. Baden LR, el Sahly HM, Essink B, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *New England Journal of Medicine*. 2021;384(5):403-416. doi:10.1056/nejmoa2035389
6. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *New England Journal of Medicine*. 2020;383(27):2603-2615. doi:10.1056/nejmoa2034577
7. Wilfredo Garcia-Beltran AF, Lam EC, St Denis K, John Iafrate A, Naranbhai V, Balazs Correspondence AB. Article Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell*. 2021;184:2372-2383.e9. doi:10.1016/j.cell.2021.03.013
8. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nature Medicine*. Published online May 17, 2021:1-7. doi:10.1038/s41591-021-01377-8
9. Feng S, Phillips Mmath DJ, White Phd T, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. doi:10.1101/2021.06.21.21258528
10. McMahan K, Yu J, Mercado NB, et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature*. 2021;590(7847):630-634. doi:10.1038/s41586-020-03041-6
11. Rogers TF, Zhao F, Huang D, et al. Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. *Science*. 2020;369(6506):956-963. doi:10.1126/science.abc7520
12. Phase 3 Prevention Trial Showed 81% Reduced Risk of Symptomatic SARS-CoV-2 Infections with Subcutaneous Administration of REGEN-COV™ (casirivimab with imdevimab) | Regeneron Pharmaceuticals Inc. Accessed June 3, 2021. https://investor.regeneron.com/news-releases/news-release-details/phase-3-prevention-trial-showed-81-reduced-risk-symptomatic-sars
13. Cohen MS, Nirula A, Mulligan MJ, et al. Effect of Bamlanivimab vs Placebo on Incidence of COVID-19 Among Residents and Staff of Skilled Nursing and Assisted Living Facilities. *JAMA*. Published online June 3, 2021. doi:10.1001/jama.2021.8828
14. Emary KRW, Golubchik T, Aley PK, et al. Efficacy of ChAdOx1 nCoV-19 (AZD1222) Vaccine Against SARS-CoV-2 VOC 202012/01 (B.1.1.7). *SSRN Electronic Journal*. Published online February 7, 2021. doi:10.2139/ssrn.3779160

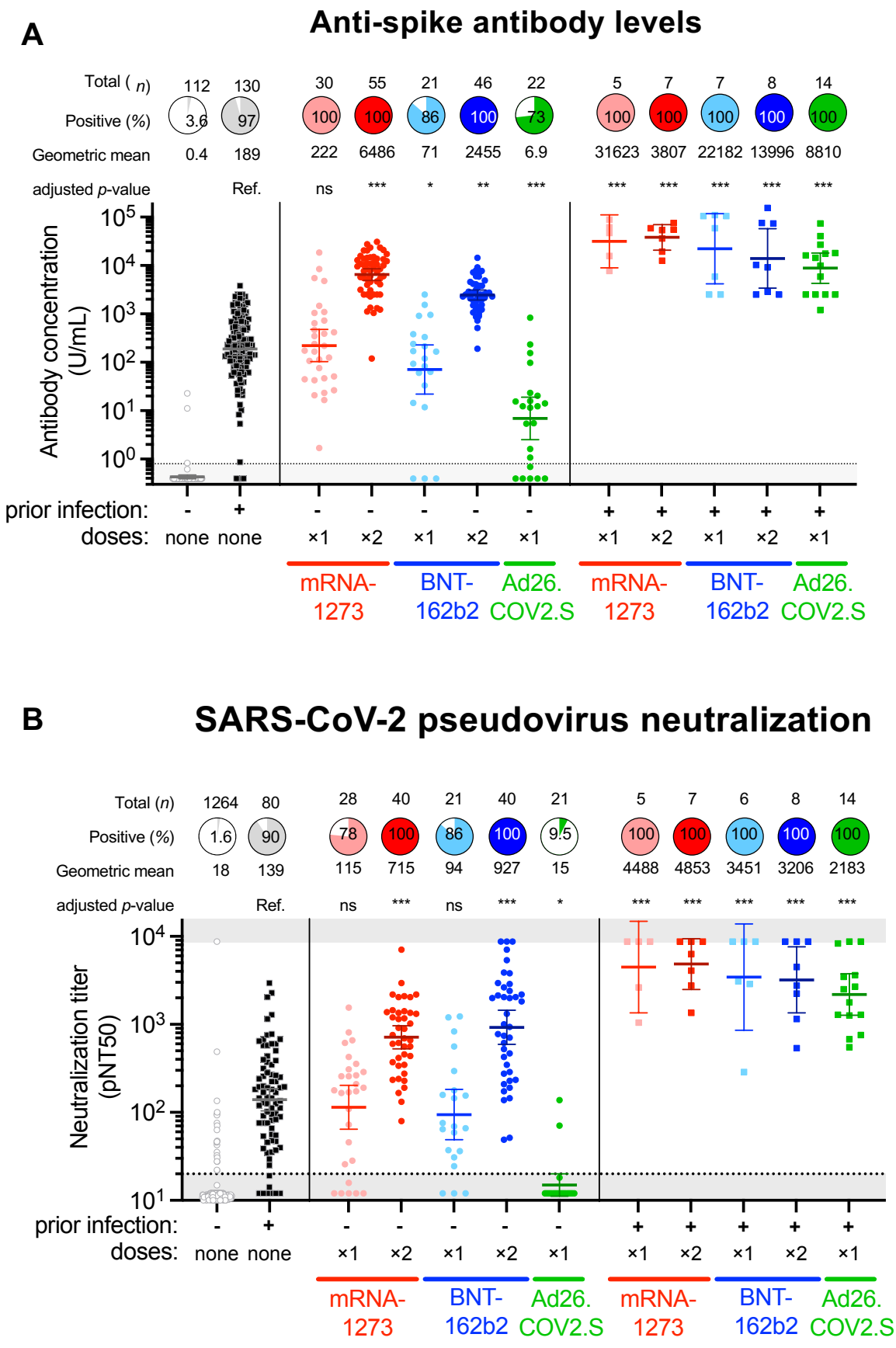
Figure Legends

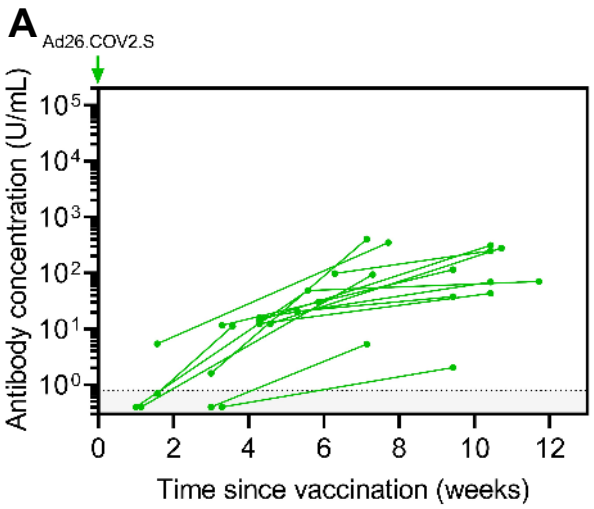
Figure 1: Humoral immunogenicity of mRNA-1273, BNT-162b2 and Ad26.COV2.S in individuals with and without prior SARS-CoV-2 infection. **Panel A** shows the quantitative SARS-CoV-2 spike IgG/A/M antibody concentration (Roche Elecsys Anti-SARS-CoV-2 assay) in U/ml of serum for 112 participants without prior infection or vaccination, 130 with prior infection and 215 following vaccination. An antibody titre of 0.8 U/ml was considered positive (dotted line). The number of donors, proportion positive and geometric mean concentration is shown above each group. **Panel B** shows pseudovirus neutralization titer 50 (pNT50, defined as the titer at which the serum achieves 50% neutralization of SARS-CoV-2 wildtype pseudovirus entry into ACE2 expressing 293T cells) for a subset of the donors above and an additional 1220 pre-pandemic controls (from Garcia-Beltran et al.[7]) used in assay validation and deriving the cutoff shown. The threshold for defining positive individuals, denoted by the dotted horizontal line, was a pNT50 of 20[7]. The number of donors, proportion positive (at a threshold of 1:20) and geometric mean titer is shown above each group. In both panel A and B, for each group the horizontal line denotes the geometric mean concentration, and whiskers extend to 95% confidence interval. Asterisks denote p-values adjusted for age, sex and duration after vaccination at time of sampling, relative to unvaccinated individuals with prior infection (see supplementary Table 2 and 4) as follows: ns not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

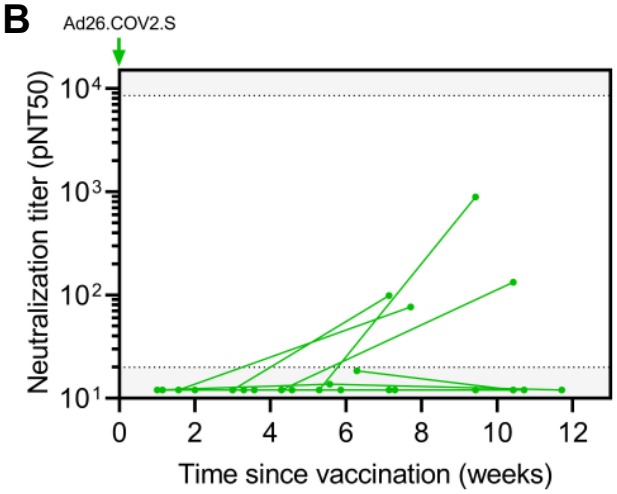
Figure 2: Follow-up measurement of response in Ad26.COV2.S recipients and kinetics of humoral responses to mRNA1273, BNT162b2 and Ad26.COV2.S SARS-CoV-2 vaccines. Longitudinal assessment of SARS-CoV-2 spike IgG/A/M antibody titers (**Panel A)** and virus neutralization **(Panel B)** in 15 Ad26CoV2.S vaccinees with baseline and repeat measures. Pooling all data in, we modelled the kinetics of antibody concentration and neutralization according to vaccine. **Panel C** shows SARS-CoV-2 spike IgG/A/M antibody levels and **Panel D** shows virus neutralization for all donors sampled after vaccination, with best-fit lines (Loess fit) shown and colored according to vaccine type, over the first 20 weeks following vaccination. In Panels C and D, the geometric mean concentration or titer, and proportion positive at threshold indicated are shown for the periods 0-2, 2-4, 4-6 and >=6 weeks in the data table insert to the right of the main figure. Each individual point and corresponding fit are colored according to

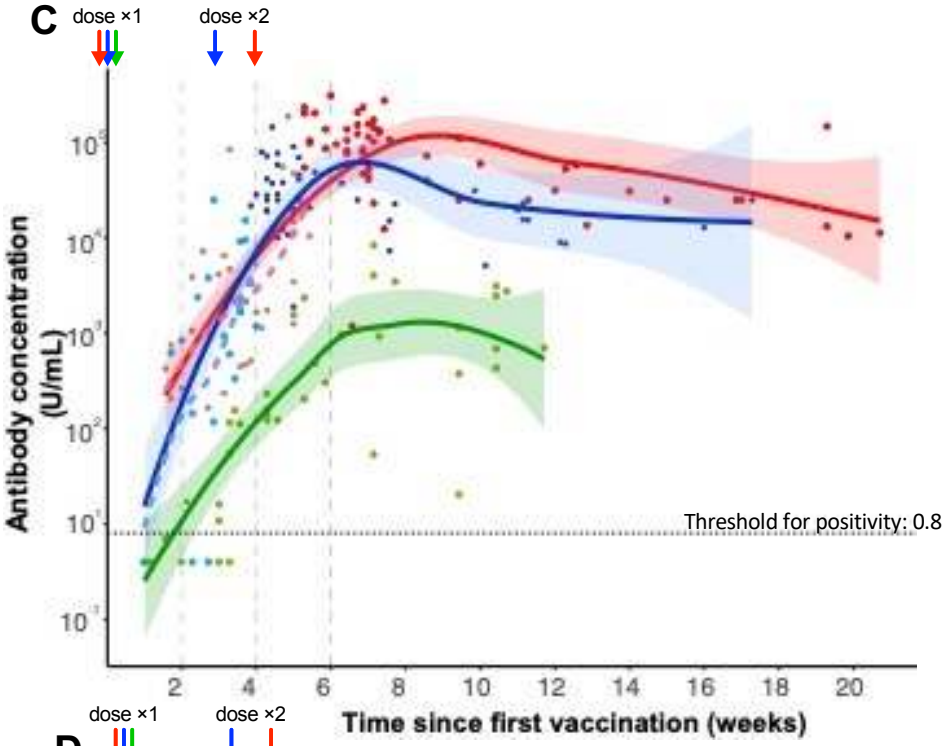
vaccine type (red mRNA-1273, blue BNT162b2 or green Ad26COV2.S), the shaded area adjacent to each line denotes the 95% confidence interval, and points are additionally shaded according to dose. Dashed lines show best-fit lines for the period after receipt of the first dose of mRNA-1273 or BNT162b2 in Panel C. In Panel D, the dotted line denotes a pNT50 threshold of 20 derived from study of pre-pandemic controls, and the upper dashed line denotes a pNT50 titer of 45.6 which represents the 20th centile of neutralization titers for unvaccinated individuals with prior infection and corresponds with 50% estimated protection in Khoury et al[8].

Figure 3: T-cell responses to spike peptides after completing SARS-CoV-2 vaccine series. Inteferon gamma ELISPOT using bulk peripheral blood mononuclear cells (PBMC) with spike peptides from SARS-CoV-2 Wuhan strain were performed. The threshold for defining positive individuals, is denoted by the dotted horizontal line at 10 SFU/10^6 PBMC. Data are normalized to background DMSO control responses. *** denotes $p<0.001$ with Kruskal-Wallis test.

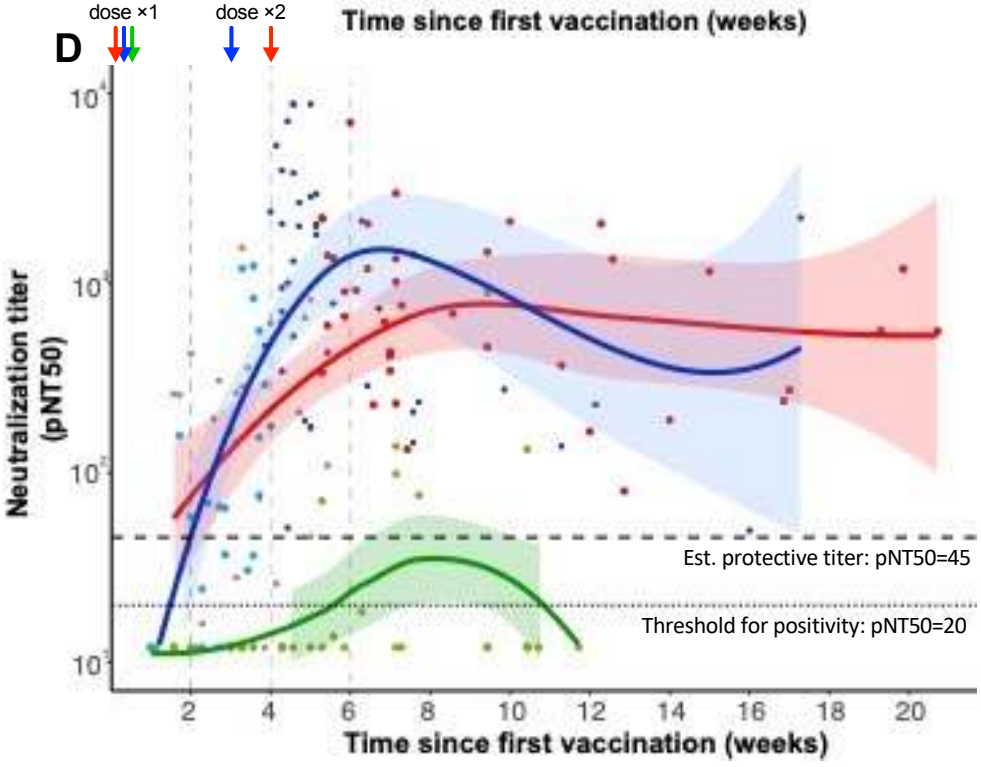








	2-4 weeks	4-6 weeks	>=6 weeks
mRNA1273			
GMC (U/mL)	115	2602	4851
proportion positive	1/15 (100%)	19/19 (100%)	32/32 (100%)
BNT162b2			
GMC (U/mL)	110	2680	1993
proportion positive	17/19 (89%)	29/29 (100%)	11/11 (100%)
Ad26.COV2.S			
GMC (U/mL)	1.7	31.2	90.6
proportion positive	5/8 (68%)	10/10 (100%)	15/15 (100%)



	2-4 weeks	4-6 weeks	>=6 weeks
mRNA1273			
GMT	104	284	654
proportion >20	11/15 (73%)	17/19 (89%)	32/32 (100%)
proportion >45	10/15 (67%)	16/19 (84%)	32/32 (100%)
BNT162b2			
GMT	108	1349	315
proportion >20	17/19 (89%)	29/29 (100%)	11/11 (100%)
proportion >45	13/19 (68%)	29/29 (100%)	11/11(100%)
Ad26.COV2.S			
GMT	<20	<20	30
proportion >20	0/8 (0%)	1/10 (10%)	5/15 (33%)
proportion >45	0/8 (0%)	1/10 (10%)	5/15 (33%)