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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

This online publication has been corrected. The corrected version first appeared at thelancet.com on August 12, 2021.

Supplement to: Borobia AM, Carcas AJ, Pérez-Olmeda M, et al. Immunogenicity and reactogenicity of BNT162b2 booster in ChAdOx1-S-primed participants (CombiVacS): a multicentre, open-label, randomised, controlled, phase 2 trial. *Lancet* 2021; published online June 25. http://dx.doi.org/10.1016/S0140-6736(21)01420-3.

SUPPLEMENTARY APPENDIX

Supplementary tables and figures

Table S1. Baseline comorbidities

	Interventional group (n=450)	Control group (n=226)	Overall (n=676)
Hypothyroidism, n (%)	21 (5)	8 (3)	29 (4)
Seasonal allergy, n (%)	21 (5)	3 (1)	24 (3)
Asthma, n (%)	20 (4)	7 (3)	27 (4)
Hypertension, n (%)	20 (4)	11 (5)	31 (5)
Hypercholesterolaemia, n (%)	12 (3)	4 (2)	16 (2)
Depression, n (%)	11 (2)	5 (2)	16 (2)
Anxiety, n (%)	10 (2)	14 (6)	24 (3)
Migraine, n (%)	9 (2)	4 (2)	13 (2)
Insomnia, n (%)	7 (1)	5 (2)	12 (2)
Arthralgia, n (%)	6 (1)	1 (0)	7 (1)
Back pain, n (%)	5 (1)	2 (1)	7 (1)
Drug hypersensitivity, n (%)	5 (1)	5 (2)	10 (1)
Rhinitis allergic, n (%)	5 (1)	2 (1)	7 (1)
TOTAL, n (%)*	352 (78)	179 (79)	532 (79)

*Only comorbidities with an absolute frequency in the interventional group of at least 5 subjects are shown.

Table S2. Summary statistics for SARS-CoV-2 anti-spike antibody response

	N	Interventional group, geometric mean [95% CI]	N	Control group, geometric mean [95% CI]	Ratio [95% CI]	p-value
RBD day 0	441	71.46[59.84;85.33]	222	89.68[68.24;117.86]	0.8[0.58;1.09]	0.1582
RBD day7	440	4353.51[3851.58;4920.85]	221	90.05[69.16;117.27]	48.34[36.15;64.65]	<0.0001
RBD day14	441	7756.68[7371.53;8161.96]	222	99.84[76.93;129.59]	77.69[59.57;101.32]	<0.0001
TrimericS day 0	441	98.4[85.69;112.99]	222	112.69[92.56;137.2]	0.87[0.69;1.11]	0.2661
TrimericS day 7	440	2246.25[2010.4;2509.78]	221	102.25[83.52;125.18]	21.97[17.45;27.66]	<0.0001
TrimericS day14	441	3684.87[3429.87;3958.83]	222	101.2[82.45;124.22]	36.41[29.31;45.23]	<0.0001

Units: BAU/mL

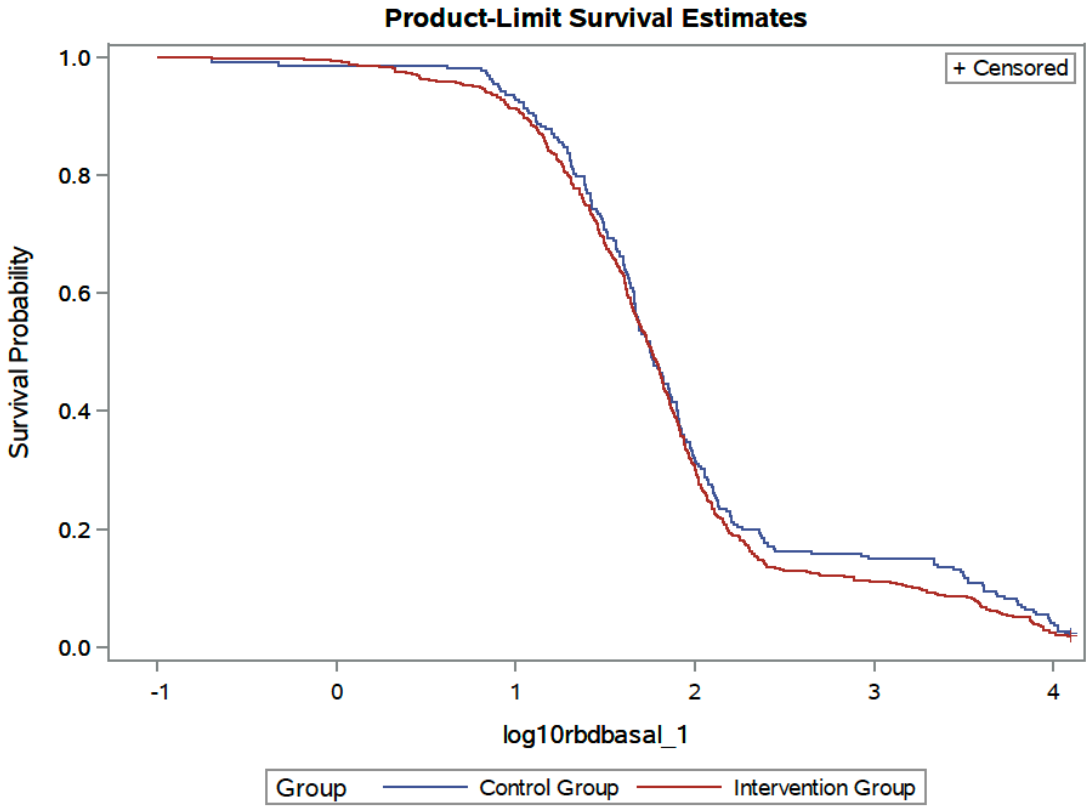
Table S3. Summary statistics for SARS-CoV-2 anti-spike antibody response by interval since ChAdOx1-S dose

	n	Interventional group, geometric mean [95% CI]	n	Control group, geometric mean [95% CI]	Ratio [95% CI]	*p*-value
8-9 WEEKS						
RBD day 0	271	108.75[91.17;129.73]	137	135.24[103.55;176.61]	0.8[0.59;1.1]	0.1696
RBD day7	270	2286.41[1977.56;2643.49]	137	123.08[93.81;161.48]	18.58[13.66;25.26]	<0.0001
RBD day14	271	3697.57[3369.11;4058.04]	137	113.15[86;148.88]	32.68[24.47;43.64]	<0.0001
TrimericS day 0	271	72.92[57.88;91.86]	137	105.84[72.69;154.11]	0.69[0.45;1.05]	0.0813
TrimericS day 7	270	4304.9[3650.46;5076.66]	137	108.88[75.36;157.31]	39.54[26.44;59.13]	<0.0001
TrimericS day14	271	7532.07[7033.12;8066.43]	137	116.57[82.23;165.25]	64.61[45.28;92.19]	<0.0001
10-11 WEEKS						
RBD day 0	170	83.89[67.14;104.81]	85	83.99[63.71;110.72]	1[0.69;1.44]	0.9948
RBD day7	170	2183.92[1837.03;2596.31]	84	75.57[56.53;101.03]	28.9[21.03;39.72]	<0.0001
RBD day14	170	3664.72[3271.5;4105.21]	85	84.53[62.34;114.63]	43.35[31.35;59.95]	<0.0001
TrimericS day 0	170	69.19[52.35;91.45]	85	68.67[46.96;100.41]	1.01[0.63;1.62]	0.9749
TrimericS day 7	170	4431.85[3699.1;5309.75]	84	66.07[46.6;93.68]	67.07[45.34;99.23]	<0.0001
TrimericS day14	170	8128.67[7544.44;8758.14]	85	77.78[52.84;114.51]	104.5[70.5;154.9]	<0.0001

Units: BAU/mL

Figure S1. Reverse cumulative distribution curves for RBD anti-spike antibodies at day 0 (a) and day 14 (b) in the interventional (red) and control (blue) groups

a)



b)

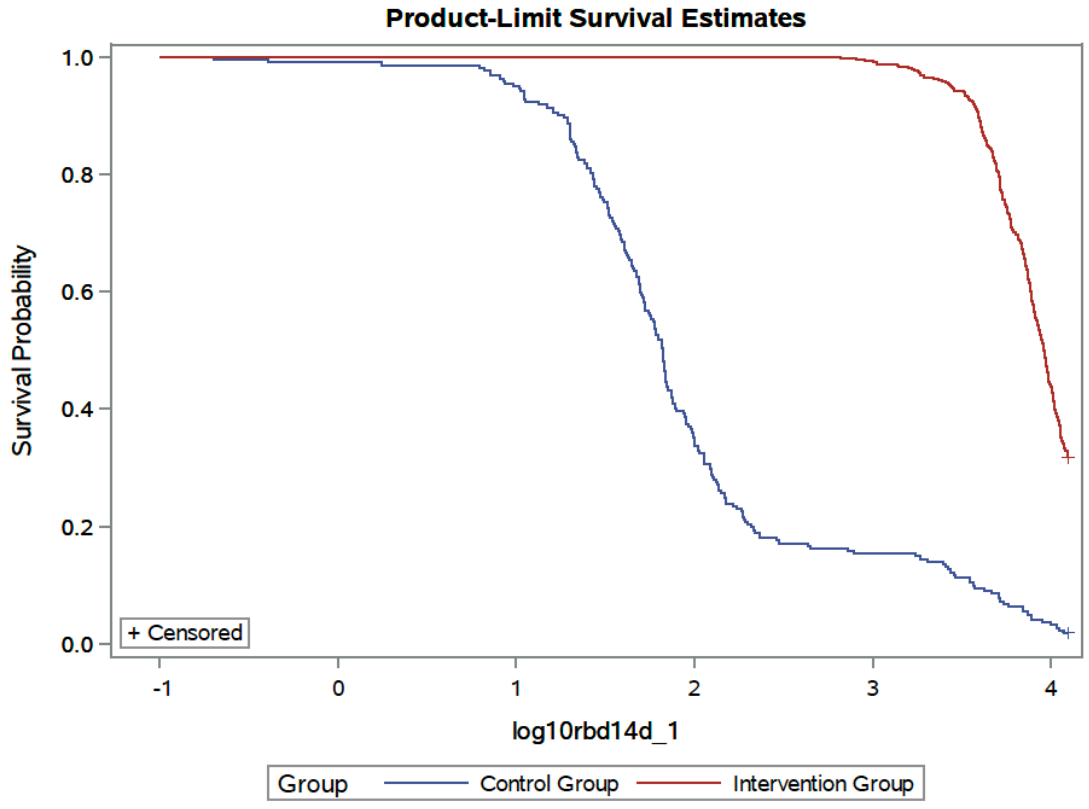
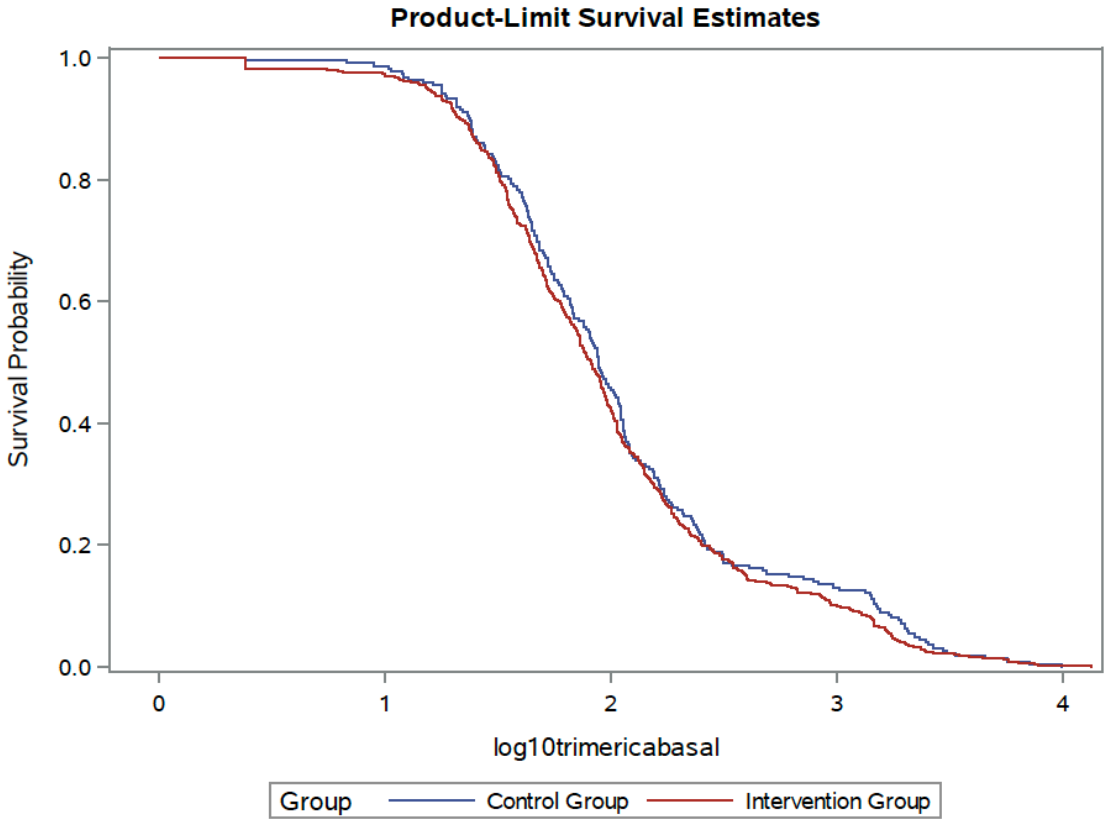


Figure S2. Reverse cumulative distribution curves for trimeric anti-spike antibodies at day 0 (a) and day 14 (b) in the interventional (red) and control (blue) groups

a)



b)

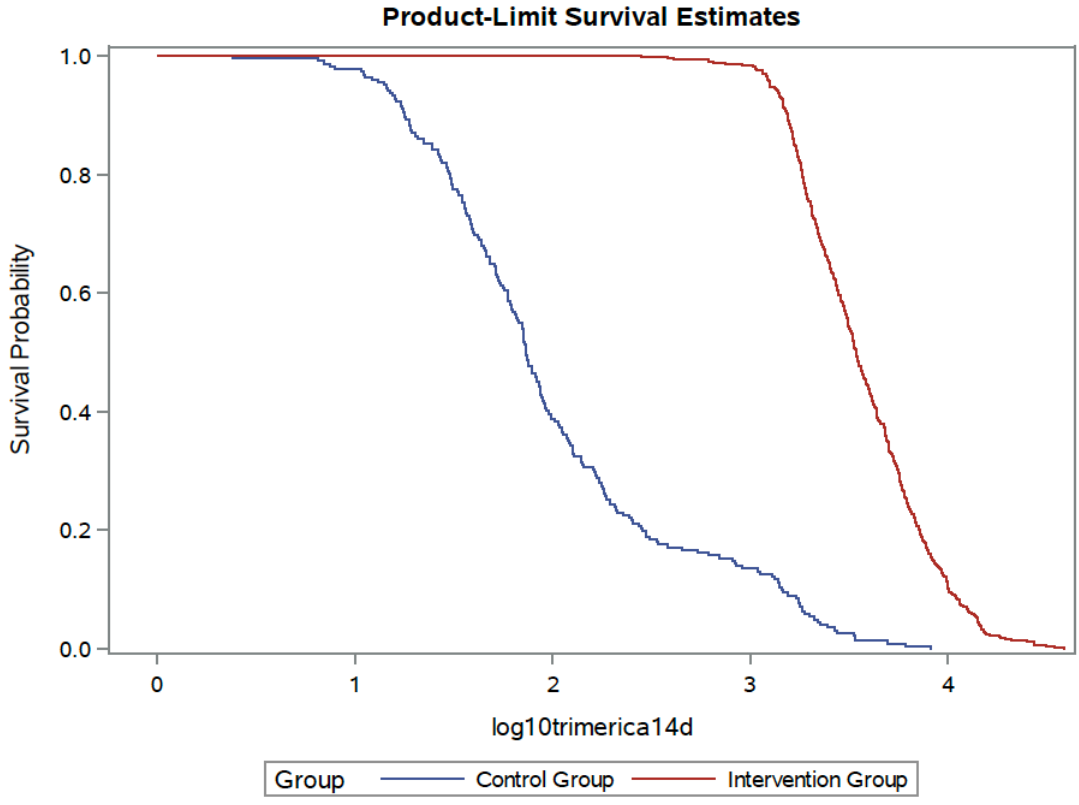


Figure S3. Relation between RBD and TrimericS measures at 14 days (Spearman correlation)

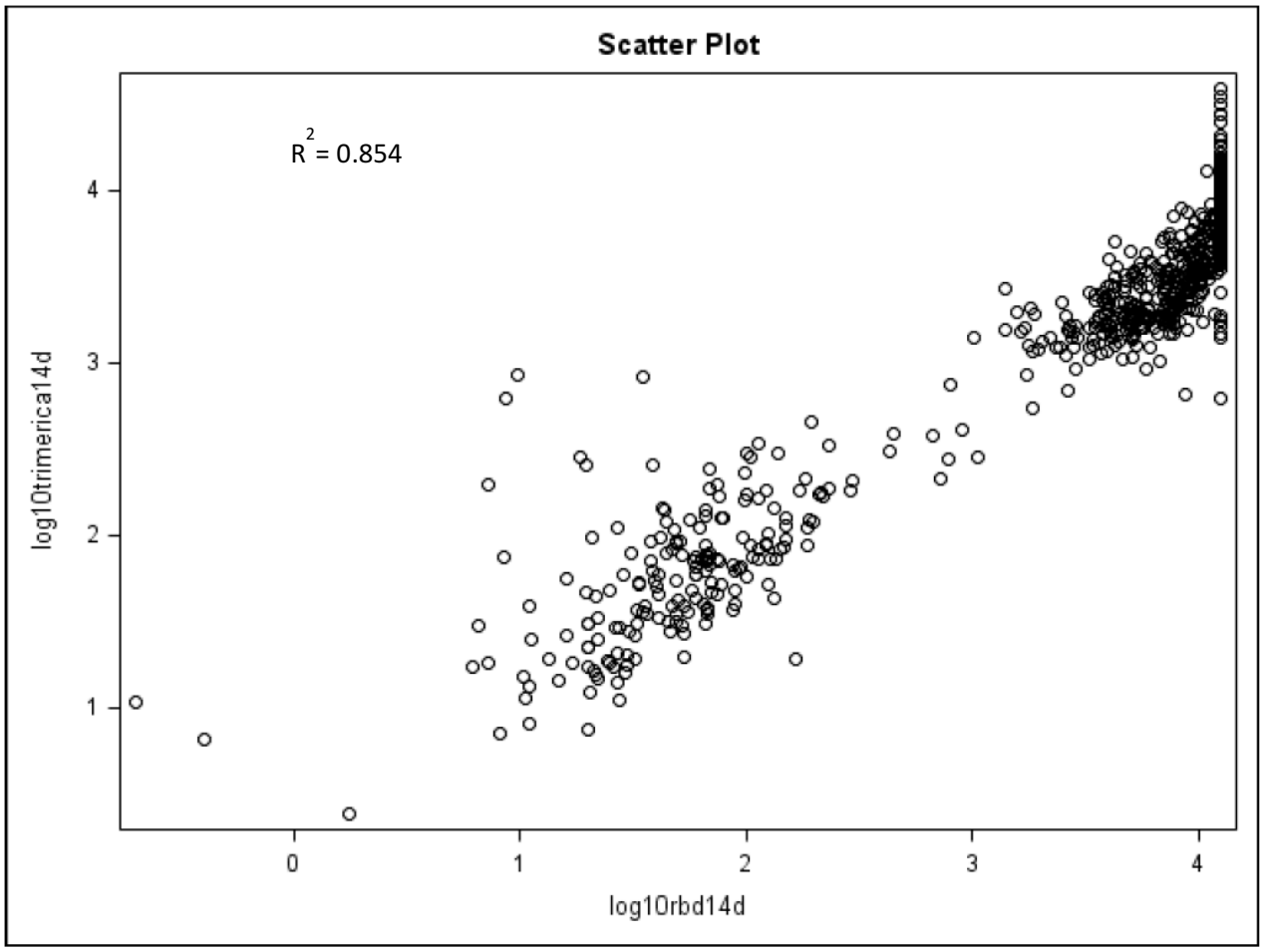


Table S4. Summary statistics for SARS-CoV-2 anti-spike antibody response by age subgroup

	n	Interventional group, geometric mean [95% CI]	n	Control group, geometric mean [95% CI]	Ratio [95% CI]	*p*-value
18-49 YEARS						
RBD day 0	287	68.73[55.57;85.01]	140	89.88[63.98;126.28]	0.76[0.52;1.12]	0.1713
RBD day7	287	4542.17[3943.71;5231.45]	140	89.63[64.96;123.68]	50.68[35.68;71.98]	<0.0001
RBD day14	287	7642.02[7156.13;8160.9]	140	96.76[69.96;133.81]	78.98[56.75;109.93]	<0.0001
TrimericS day 0	287	94.52[80.05;111.6]	140	112.68[88.74;143.09]	0.84[0.63;1.12]	0.2336
TrimericS day 7	287	2361.46[2070.09;2693.85]	140	101.05[79.17;128.97]	23.37[17.72;30.82]	<0.0001
TrimericS day14	287	3576.28[3283.46;3895.23]	140	101.01[78.71;129.62]	35.41[27.21;46.07]	<0.0001
50-59 YEARS						
RBD day 0	154	76.83[55.73;105.91]	82	89.34[55.94;142.68]	0.86[0.49;1.5]	0.5920
RBD day7	153	4020.5[3181.95;5080.03]	81	90.79[56.92;144.81]	44.29[26.32;74.51]	<0.0001
RBD day14	154	7974.98[7361.48;8639.61]	82	105.34[67.38;164.71]	75.7[48.09;119.17]	<0.0001
TrimericS day 0	154	106.05[82.68;136.04]	82	112.7[79.44;159.89]	0.94[0.62;1.44]	0.7777
TrimericS day 7	153	2045.09[1669.12;2505.76]	81	104.36[72.58;150.06]	19.6[12.95;29.66]	<0.0001
TrimericS day14	154	3896.11[3419.07;4439.71]	82	101.53[70.63;145.95]	38.37[26.12;56.38]	<0.0001

Units: BAU/mL

Table S5. Summary statistics for SARS-CoV-2 anti-spike antibody response by gender

	n	Interventional group, geometric mean [95% CI]	n	Control group, geometric mean [95% CI]	Ratio [95% CI]	*p*-value
MALE						
RBD day 0	189	62.04[47.21;81.52]	98	96.58[61.93;150.62]	0.64[0.39;1.05]	0.0787
RBD day7	189	3387.65[2737.79;4191.77]	98	93.95[60.97;144.77]	36.06[22.3;58.3]	<0.0001
RBD day14	189	7219.19[6683.76;7797.52]	98	106.98[70.66;161.97]	67.48[44.27;102.86]	<0.0001
TrimericS day 0	189	84.73[68.44;104.9]	98	113.61[82.97;155.57]	0.75[0.51;1.08]	0.1211
TrimericS day 7	189	1534.95[1285.54;1832.75]	98	103.13[74.75;142.29]	14.88[10.32;21.47]	<0.0001
TrimericS day14	189	2954.66[2677.37;3260.68]	98	97.75[70.78;134.99]	30.23[21.58;42.34]	<0.0001
FEMALE						
RBD day 0	252	79.45[62.87;100.4]	124	84.58[59.85;119.53]	0.94[0.62;1.42]	0.7649
RBD day7	251	5258.61[4573.38;6046.51]	123	87.07[62.49;121.31]	60.4[42.17;86.5]	<0.0001
RBD day14	252	8185.9[7651.35;8757.8]	124	94.54[67.47;132.47]	86.59[61.39;122.11]	<0.0001
TrimericS day 0	252	110.08[91.82;131.97]	124	111.97[86.86;144.32]	0.98[0.72;1.34]	0.9149
TrimericS day 7	251	2992.1[2623.1;3413.01]	123	101.55[78.17;131.93]	29.46[22;39.46]	<0.0001
TrimericS day14	252	4348.68[3946.56;4791.78]	124	104.02[79.59;135.94]	41.81[31.46;55.55]	<0.0001

Units: BAU/mL

Figure S4. Reverse cumulative distribution curves for neutralizing antibodies at day 0 in the interventional group by interval since ChAdOx1-S dose

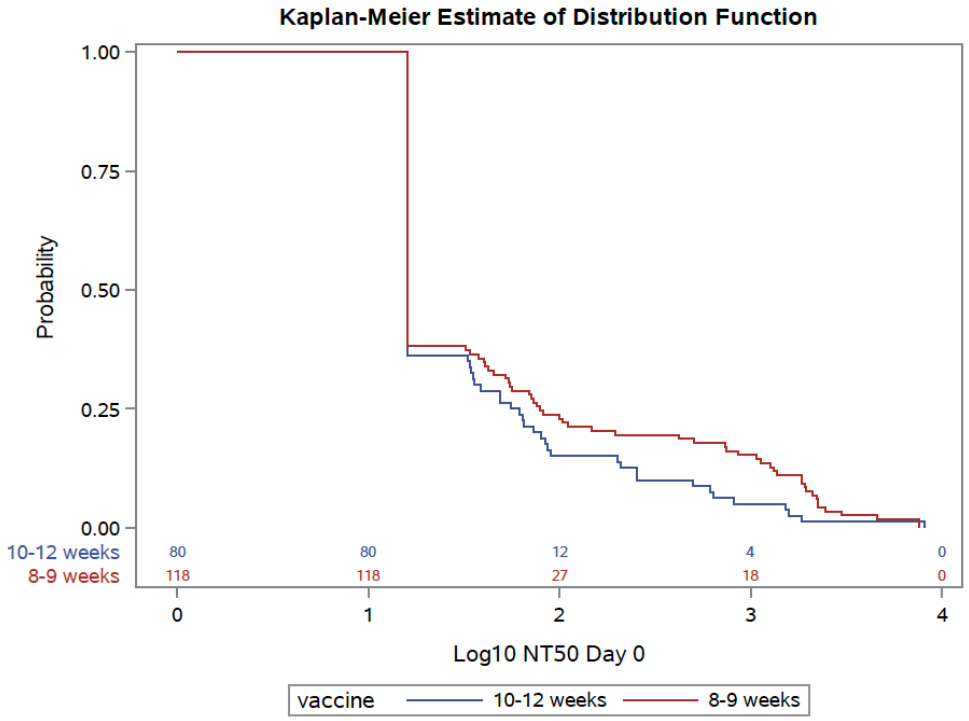


Table S6. Neutralizing activity (NT50) by categories

	Interventional group (n=129)		Control group (n=69)*	
	Day 0	**Day 14**	**Day 0**	**Day 14**
Negative (<32), n (%)	85 (65.9)	0 (0)	39 (56.5)	48 (69.6)
>32/<60, n (%)	11 (8.5)	0 (0)	9 (13)	6 (8.7)
>60/<300, n (%)	13 (10.1)	3 (2.3)	10 (14.5)	4 (5.8)
>300/<1000, n (%)	6 (5.7)	29 (22.5)	3 (4.3)	3 (4.3)
>1000, n (%)	14 (10.8)	97 (75.2)	8 (11.6)	8 (11.6)

* Two participants were excluded because of lacking day-14 blood sample

Table S7. Neutralizing activity (NT50) in the interventional group (n=129) by baseline NT50

	N	Day 0, geometric mean [95% CI]	Day 14, geometric mean [95% CI]
Negative (<32)*	85	16 [16-16]	1356 [1153-1594]
>32/<60	11	41 [36-47]	2704 [1940-3768]
>60/<300	13	97 [76-124]	2253 [1473-3445]
>300/<1000	6	558 [452-690]	4308 [2661-6975]
>1000	14	2189 [1444-3318]	6904 [4038-11803]

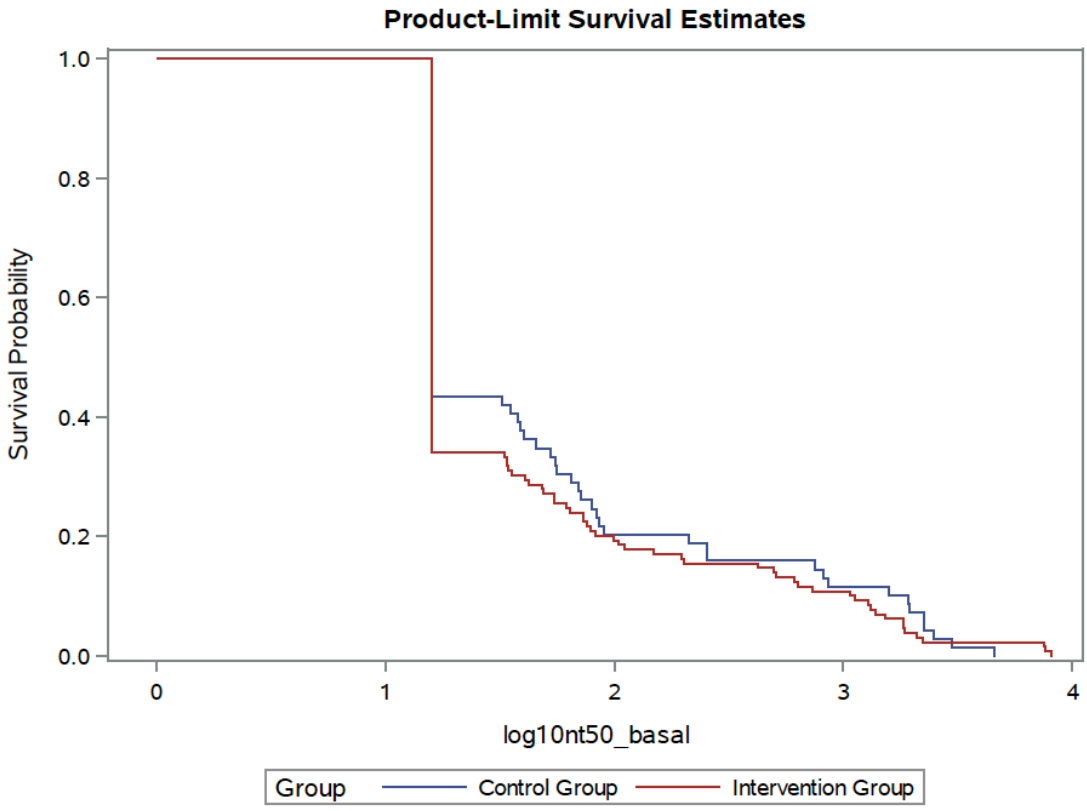
* Patients below the detection threshold (32) were given 16 value

Table S8. Summary statistics for neutralising antibody response

	N	Interventional group, geometric mean [95% CI]	N	Control group, geometric mean [95% CI]	Ratio [95% CI]	p-value
NT50 day 0	129	41.84 [31.28;55.96]	69	50.84 [33.56;76.99]	0.82 [0.5;1.35]	0.4407
NT50 day 14	129	1905.69 [1625.65;2233.98]	69	41.81 [27.18;64.32]	45.57 [28.84;72.03]	<0.0001

Figure S5. Reverse cumulative distribution curves for neutralizing antibodies at day 0 (a) and day 14 (b) in the interventional (red) and control (blue) groups

a)



b)

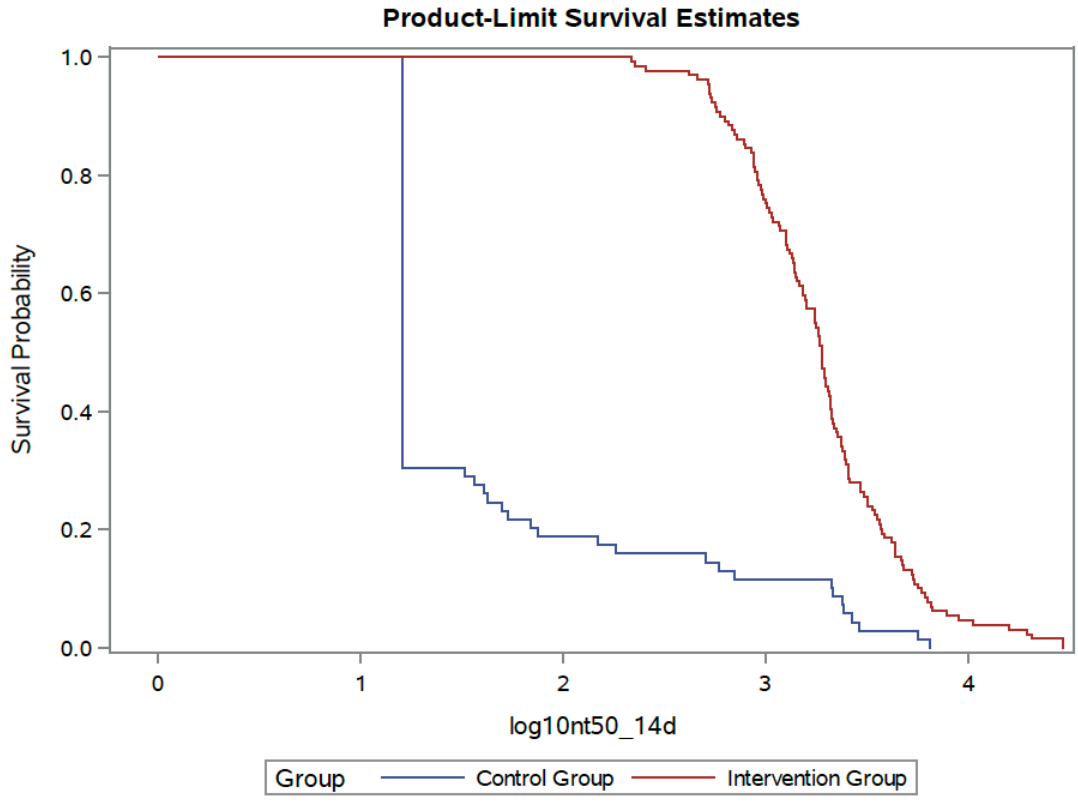


Figure S6. Reverse cumulative distribution curves for neutralizing antibodies at day 14 in the interventional group by baseline NT50

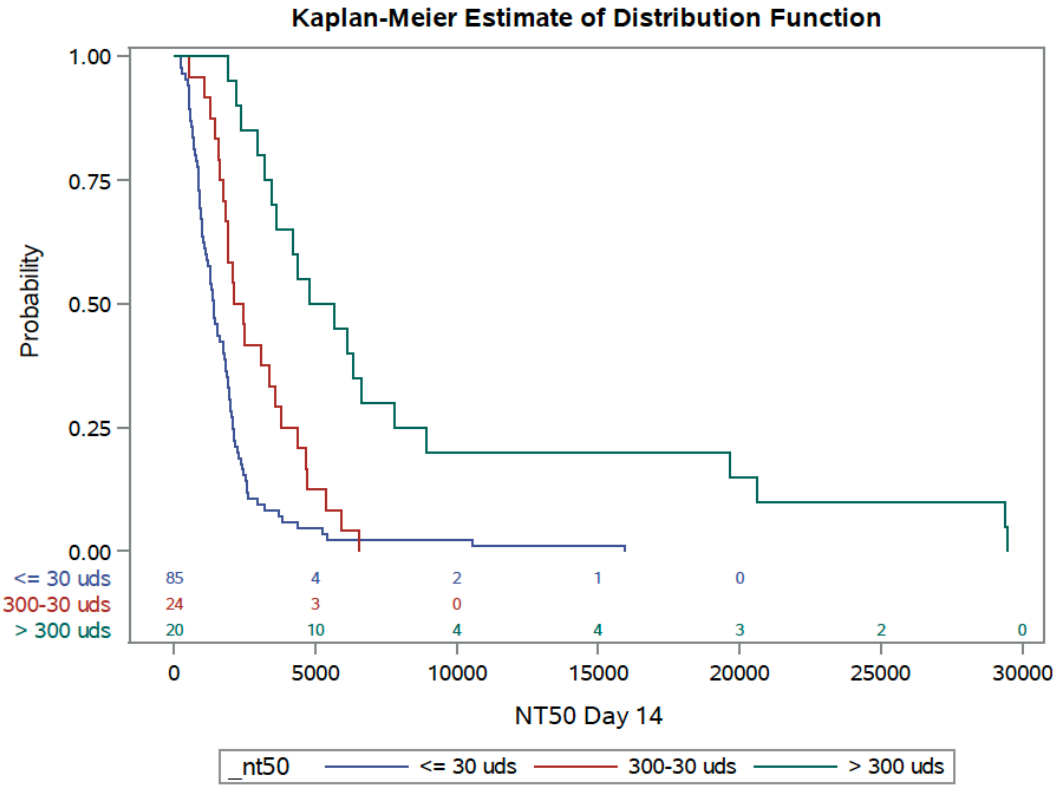
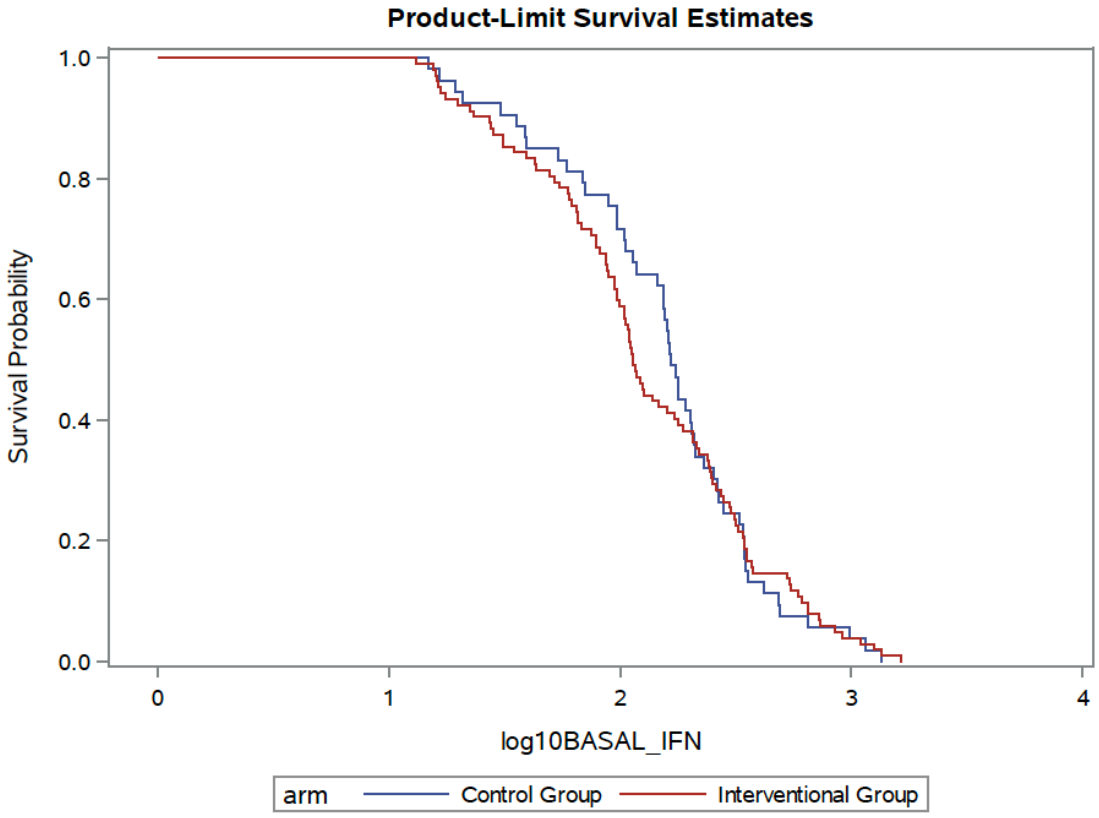


Figure S7. Reverse cumulative distribution curves for IFN-gamma concentration at day 0 (a) and day 14 (b) in the interventional (red) and control (blue) groups

(a)



(b)

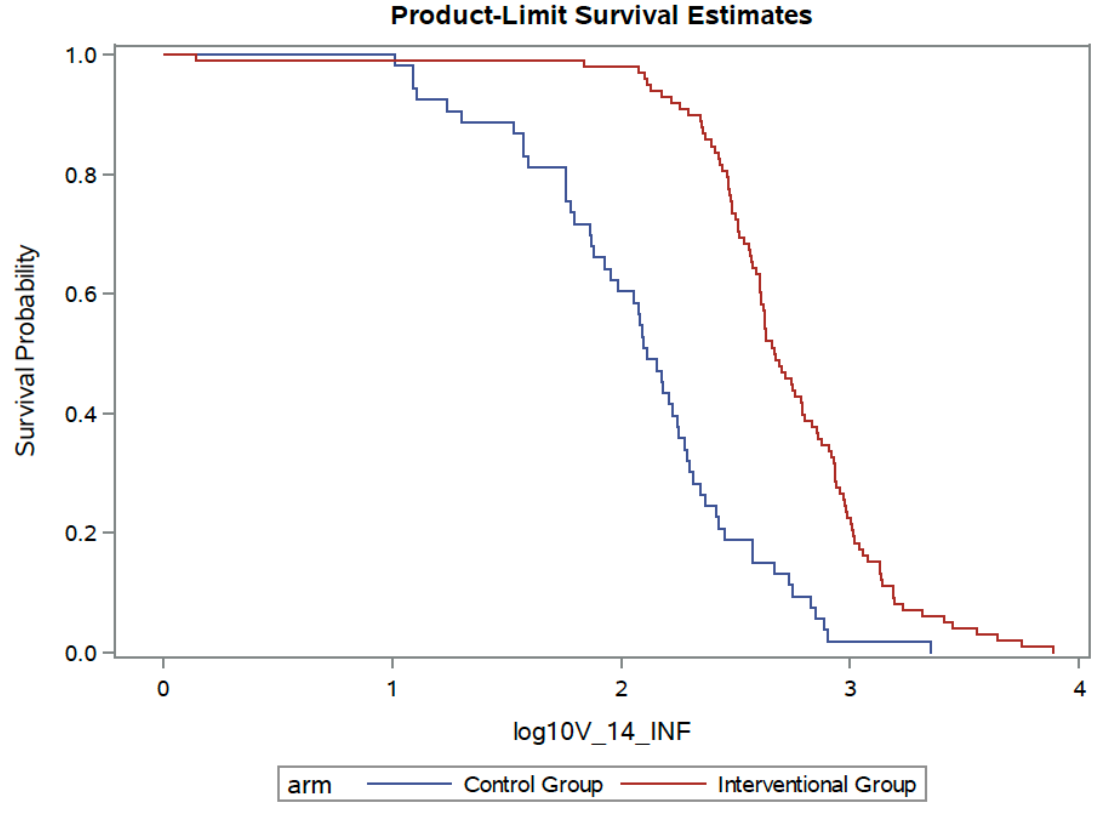


Table S9. Local and systemic reactions in first 7 days after BNT162b2 vaccination

	Interventional Group (n=448)
Chills, n (%)	114 (25.4)
Cough, n (%)	33 (7.4)
Headache, n (%)	199 (44.4)
Injection site discomfort, n (%)	395 (88.2)
Injection site hardness, n (%)	159 (35.5)
Injection site urticaria, n (%)	67 (15.0)
Vaccination site erythema, n (%)	139 (31.0)
Myalgia, n (%)	194 (43.3)
Arthralgia, n (%)	155 (34.6)
Injection site pruritus, n (%)	49 (10.9)
Malaise, n (%)	187 (41.7)
Pyrexia, n (%)	11 (2.5)
Nausea, n (%)	49 (10.9)
Pruritus, n (%)	9 (2.0)
Vomiting, n (%)	4 (0.9)
Rash, n (%)	6 (1.3)
Injection site pustule, n (%)	1 (0.2)

Table S10. Local and systemic reactions in first 7 days after BNT162b2 vaccination, by gender and by age group

	By gender			By age group			Overall
	Female (n=255)	Male (n=193)	*p*-value	18-49 years (n=292)	50-59 (years) (n=156)	*p*-value	(n=448)
Injection site discomfort, n (%)	227 (89.0)	168 (87.0)	0.5220	261 (89.4)	134 (85.9)	0.2764	395 (88.2)
Injection site hardness, n (%)	104 (40.8)	55 (28.5)	0.0071	104 (35.6)	55 (35.3)	0.9395	159 (35.5)
Injection site pruritus, n (%)	32 (12.5)	17 (8.8)	0.2090	28 (9.6)	21 (13.5)	0.2109	49 (10.9)
Injection site pustule, n (%)	1 (0.4)	0	0.9999	0	1 (0.6)	0.3482*	1 (0.2)
Injection site urticaria, n (%)	52 (20.4)	15 (7.8)	0.0002	40 (13.7)	27 (17.3)	0.3075	67 (15.0)
Arthralgia, n (%)	99 (38.8)	56 (29.0)	0.0307	99 (33.9)	56 (35.9)	0.6726	155 (34.6)
Chills, n (%)	75 (29.4)	39 (20.2)	0.0268	75 (25.7)	39 (25.0)	0.8740	114 (25.4)
Cough, n (%)	19 (7.5)	14 (7.3)	0.9370	19 (6.5)	14 (9.0)	0.3408	33 (7.4)
Headache, n (%)	125 (49.0)	74 (38.3)	0.0243	138 (47.3)	61 (39.1)	0.0978	199 (44.4)
Malaise, n (%)	122 (47.8)	65 (33.7)	0.0026	128 (43.8)	59 (37.8)	0.2187	187 (41.7)
Myalgia, n (%)	130 (51.0)	64 (33.2)	0.0002	126 (43.2)	68 (43.6)	0.9288	194 (43.3)
Nausea, n (%)	36 (14.1)	13 (6.7)	0.0132	34 (11.6)	15 (9.6)	0.5122	49 (10.9)
Pruritus, n (%)	4 (1.6)	5 (2.6)	0.5083*	8 (2.7)	1 (0.6)	0.1713*	9 (2.0)
Pyrexia, n (%)	8 (3.1)	3 (1.6)	0.3644*	6 (2.1)	5 (3.2)	0.5258*	11 (2.5)
Rash, n (%)	6 (2.4)	0	0.0393*	3 (1.0)	3 (1.9)	0.4240*	6 (1.3)
Vaccination site erythema, n (%)	90 (35.3)	49 (25.4)	0.0248	87 (29.8)	52 (33.3)	0.4405	139 (31.0)
Vomiting, n (%)	4 (1.6)	0	0.1377*	2 (0.7)	2 (1.3)	0.6131*	4 (0.9)

By default, Chi-Square test. (*) Fisher´s Exact Test – 25% of the cells have expected counts less than 5.

Supplementary Methods

Table S11. Number of subjects with full data for each variable analysed

Variable	Interventional group (n=441)	Control group (n=222)
TrimericS day 0	441	222
TrimericS day 7	440	221
TrimericS day14	441	222
RBD day 0	441	222
RBD day7	440	221
RBD day14	441	222
NT50 day 0	129	69
NT50 day14	129	69
IFN day 0	101	53
IFN day14	98	53
IL2 day 0	101	53
IL2 day14	98	53

Pseudo-virus neutralization assay

Cell lines

The Vero E6 (African green monkey kidney) cell line was kindly provided by Dr. A. Alcami (CBM Severo Ochoa, Madrid). HEK-293T (National Institute for Biological Standards and Control (NIBSC]) and Vero E6 cells were cultured in DMEM supplemented with 10% FCS, 2 mM L-glutamine and 100 units/ml penicillin and streptomycin (Lonza).

Pseudo-virus generation and neutralization assay

The spike of the SARS-CoV-2 virus was generated (GeneArt Gene Synthesis, ThermoFisher Scientific) from the codon-optimized sequence obtained by Ou et al. (1) and inserted into pcDNA3.1D/V5-His-TOPO (pcDNA3.1-S-CoV2Δ19-G614). This plasmid presents the mutation D614G and a deletion in the last 19 aa from the original spike. The pcDNA-VSV-G plasmid contains the cDNA encoding the vesicular stomatitis virus G protein and was obtained from Dr. Arenzana-Seisdedos (Institute Pasteur, Paris, France).

NL4.3 pseudotypes were generated with the previously described plasmid pNL4-3ΔenvRen (2). Briefly, Renilla luciferase reporter pseudovirus were prepared by co-transfecting HEK-293T cells with pNL4-3ΔenvRen backbone and viral envelope protein expression plasmid pcDNA3.1-S-CoV2Δ19-G614 or pcDNA-VSV-G using the calcium phosphate method. The medium was changed 18 hours after transfection, and 48 hours post-transfection cell culture supernatants were harvested, clarified by centrifugation at 500 x g for 5 min, and frozen at -80 °C. The amount of p24 antigen in the supernatants was quantified by electrochemiluminescence Immunoassay (Roche Diagnostic).

To measure neutralization activity of plama from donors, four-fold serial dilutions of heat-inactivated sera (1/32-1/131072) were preincubated with titrated pseudoviruses (10 ng p24 Gag/well) for 1 hour at 37 °C. Thereafter, 100 µl of the mixture was added to Vero E6 cells plated at 5 x 103 cells/well in 100 µl medium in 96-well plates the previous day. The culture medium was refreshed after 16 hours. At 48 h post-infection, cells were lysed, and viral infectivity was assessed by measuring luciferase activity (Renilla Luciferase Assay, Promega) using a 96-well plate luminometer "LB 960 Centro XS³" (Berthold). The titers of neutralizing antibodies were calculated as 50% inhibitory dose (neutralizing titer 50, NT50), expressed as the highest dilution of plasma which resulted in a 50% reduction of luciferase activity compared to control without plasma. Sigmoid curves were generated and NT50s were calculated by non-linear regression using GraphPad Prism version 9.0.1 (GraphPad Software, Inc.). VSV-G pseudoviruses were used as a specificity control virus in neutralization testing.

1. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, Guo L, Guo R, Chen T, Hu J, Xiang Z, Mu Z, Chen X, Chen J, Hu K, Jin Q, Wang J, Qian Z. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun. 2020;11: 1620.
2. Garcia-Perez J, Sanchez-Palomino S, Perez-Olmeda M, Fernandez B, Alcami J. A new strategy based on recombinant viruses as a tool for assessing drug susceptibility of human immunodeficiency virus type 1. J Med Virol. 2007 Feb;79(2):127-37.

Cellular immune response quantification

SARS-CoV-2 Peptide pools

SARS-CoV-2 peptide pools of Prot S/S1/S+ from Miltenyi, were used as recently reported (1).

Whole blood culture with SARS-CoV-2 peptide pools

320 µl of whole blood drawn on the same day were mixed with 80 µl RPMI and stimulated with pools of SARS-CoV-2 peptides (S; 2 µg/ml) or DMSO control. After 15 hours of culture, the supernatant (plasma) was collected and stored at -80ºC until quantification of cytokines are recently described (2).

Cytokine quantification and analysis

Cytokine concentrations in the plasma were quantified using Ella with microfluidic multiplex cartridges measuring IFN-γ following the manufacturer's instructions (ProteinSimple, San Jose, California). The level of cytokines present in the plasma of DMSO controls was subtracted from the corresponding peptide pool stimulated samples.

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Study Protocol

Protocol Title:

A Phase 2, Comparative, Randomised, Adaptive Trial to Evaluate the safety and immunogenicity of one dose of COMIRNATY® in subjects that had received one dose of VAXZEVRIA®

Protocol Version and Date: 1.2 / May 21, 2021

(Includes non-substantial modification number 2)

Protocol Code: CombiVacS

Brief Title: Vaccination with COMIRNATY in subjects with a VAXZEVRIA first dose.

CombiVacS is a phase 2 randomized, adaptive trial to evaluate the immunogenicity and safety of one dose of mRNA COVID19 vaccine (COMIRNATY – Pfizer) in subjects having received a single previous dose of a viral vector COVID19 vaccine (VAXZEVRIA – AstraZeneca)

Study Phase: 2

Sponsor Name:
Instituto de Salud Carlos III (ISCIII)

Regulatory Agency Identifier Number:

EudraCT 2021-001978-37

Approval Date:

Sponsor Signatory: _____ _____

Date

1. Protocol Summary

1.1. Synopsis

Name of Sponsor	**Instituto de Salud Carlos III (ISCIII)**
Protocol Number/Title:	A Phase 2, Comparative, Randomised, Adaptive Trial to Evaluate the safety and immunogenicity of one dose of COMIRNATY in subjects that had received one dose of VAXZEVRIA
Indication:	Covid-19 prevention
Hypothesis	Immunogenicity in patients who have already received one dose of VAXZEVRIA will be higher when it is followed by one dose of COMINARTY administered 8-12 weeks later.
Trial Objectives:	**Primary Immunogenicity Objective:** • To assess the humoral immune response against SARS-CoV-2, 14 days after a vaccination with COMIRNATY in subjects that had received a previous single dose of VAXZEVRIA (Group 1) as compared with no dosing (Group 2). **Secondary Immunogenicity Objectives:** • To assess the humoral immune response against SARS-CoV-2 28 days after a vaccination with COMIRNATY in subjects that received a previous single dose of VAXZEVRIA . • To assess the long-term (up to 1 year) humoral immune response against SARS-CoV-2 of a dose of COMIRNATY in subjects that received a previous single dose of VAXZEVRIA. • To assess the humoral immune response against viral variants of SARS-CoV-2, 14 and 28 days after a dose of COMIRNATY in subjects that received a previous single dose of VAXZEVRIA. **Exploratory objective:** • To evaluate the relationship between the immune response measured as NAV (Neutralizing antibodies) and antibodies against SARS-CoV-2 spike protein measured by immunoassay. **Secondary efficacy objectives:** • To assess the occurrence of symptomatic molecularly confirmed COVID-19 and severity of COVID-19 signs and symptoms after the administration of a dose of COMIRNATY in subjects that received a prior single dose of VAXZEVRIA. **Primary Safety Objectives:** • To evaluate the safety of a dose of COMIRNATY in subjects that received a previous single dose of VAXZEVRIA.
Trial Design Overview:	This is a randomized, non-blinded, controlled, adaptive, multicenter, Phase II study in subjects aged ≥18 years and in good health or stable clinical situation that have received a previous single dose of VAXZEVRIA. This is a phase 2 adaptive trial developed to evaluate the immunogenicity of a dose of COMIRNATY after a previous single dose of VAXZEVRIA. A stratification will be made based on the following factors: study site, sex and age. This protocol allows to test the immunogenicity and safety of a heterologous vaccination strategy after a previous single dose of VAXZEVRIA. Subjects will be randomized to immediately receive or not a dose of COMIRNATY in a ratio of 2:1. If primary analysis at 14 days confirms the starting hypothesis, subjects randomized to no vaccination will be considered for administration of one dose of COMINARTY at day 28th according to Public Health Department of the

	Ministry of Health recommendations on heterologous vaccination. If vaccinated, subjects will be followed at the time points defined in the flowchart. In case the primary analysis does not confirm the starting hypothesis, subjects will be followed at the time points defined in the flow-chart without administration of COMIRNATY. In case Public Health Department gave instructions to give the second dose of VAXZEVRIA to those subjects having received the first dose, subject will be followed as indicated in flowchart of Group 2. Other heterologous vaccination strategies could be incorporated if deemed necessary for public health reasons. This could include the use of different vaccination strategies including those already marketed vaccines for comparative assessment of their safety and efficacy on SARS-CoV-2 and its variants. The different intervention arms planned (and its corresponding control arm) can be carried-out in different trial sites, thus not all trial sites must participate in all arms. See trial schematic for more details.
Intervention and control arms	Intervention arm (Group 1): administration of a COMINARTY dose. Control arm (Groups 2): no administration of COMINARTY dose.
Randomization and blinding	**Randomization**. A central randomization stratified by study site and age will be used. Randomization will be in a ratio of 2:1 **Blinding.** No blinding is planned in this trial
Participant Population:	**Inclusion criteria** 1. Adult subjects (18 years old) having received prior VAXZEVRIA vaccination between 8 and 12 weeks before the screening visit. 2. Participants must provide consent indicating that he or she understands the purpose, procedures and potential risks and benefits of the study, and is willing to participate in the study. 3. Subjects in good health or stable clinical situation. 4. Participant is willing and able to adhere to the procedures specified in this protocol. **Exclusion criteria** 1. Participant has a clinically significant acute illness (this does not include minor illnesses such as diarrhea or mild upper respiratory tract infection) or temperature ≥38.0ºC within 24 hours prior to the planned dose of study vaccine. 2. Participant has a known or suspected allergy or history of anaphylaxis or other serious adverse reactions to COMIRNATY excipients. 3. Subjects with any contraindication to the administration of COMIRNATY, included pregnancy. 4. Subjects with prior documented COVID19 since VAXZEVRIA vaccination. 5. Subjects have symptoms or signs compatible with COVID19. 6. Subjects participating in a clinical trial in the last three months. 7. Any condition or situation precluding or interfering the compliance with the protocol.

	Number of participants: The total planned number of patients to be included is 600 (400 in COMINARTY arm and 200 in no vaccination arm).
Medicinal Product, Dose and Mode of Administration:	1) COMIRNATY at standard dose will be administered at the standard dose defined in the summary of product characteristics in subjects randomized to receive the dose immediately or in control arm if primary analysis at 14 days confirms the starting hypothesis and no recommendations against are issued by the Public Health Department.

Primary immunogenicity endpoint:

- Serological response to vaccination (antibodies against SARS-CoV-2 spike protein) as measured by immunoassay, 14 days after vaccine dose (Group 1) or randomization (Group 2) .

Secondary immunogenicity endpoint:

- Serological response to vaccination (antibodies against SARS-CoV-2 spike protein) as measured by immunoassay, 28 days after vaccine dose (Group 1) or randomization (Group 2) .
- SARS-CoV-2 neutralization as measured by virus neutralization assay, 14 and 28 days after vaccine dose (Group 1) or randomization(Group 2) .
- Serological response to vaccination (antibodies against SARS-CoV-2 spike protein) as measured by immunoassay at 3, 6 and 12months after randomization.
- Neutralizing antibody titers at 3, 6 and 12 months after randomization.

Secondary efficacy endpoints:

- The number of participants with molecularly confirmed COVID-19.
- Presence and severity of COVID-19 signs and symptoms as measured by Symptoms of Infection with Coronavirus-19 (SIC).

Primary safety endpoints:

- Solicited local and systemic adverse events (AEs) for a period of 7 days after vaccination.
- Unsolicited local and systemic adverse events (AEs) for a period of 28 days after vaccination
- Serious adverse events (SAEs) throughout the study (from randomization until end of the study).
- Medically-attended adverse events (MAAEs) from the day of vaccination until 6 months after the last vaccination.
- Abnormal laboratory parameters at 1 month visit after COMIRNATY vaccination.

Statistical Analysis.

Primary immunogenicity endpoint is the titer of antibodies binding to the SARS-CoV-2 S protein on14^{th} day measured by immunoassay.

Secondary immunogenicity endpoint is NAV (FRNT50 GMT, which represent the reciprocal dilution of serum that neutralizes 50% of the input virus expressed as the geometric men) evaluated in the randomization visit (previously COMIRNATY vaccination) and on 14, 28 days and 3, 6 and 12 months after dosing.

Primary statistical analysis will be performed on day 14^{th} after randomization.

Main comparisons are:

- Immune response measured by immunoassay on 14 days after the randomization (±2 days).

Other comparisons will include:

- Immune response (antibodies against SARS-CoV-2 spike protein) measured by immunoassay at 7 days after the randomization (±2 days).
- Immune response (antibodies against SARS-CoV-2 spike protein) measured by immunoassay at 28 days after the randomization (±2 days)
- Immune response measured by NAV at 14 days after the randomization (±2 days).
- Immune response measured by NAV at 28 days after the randomization (±2 days)
- Correlation of immune response measured by NAV and immunoassay (antibodies against SARS-CoV-2 spike protein).

- Change in immune response from baseline (randomization visit) to 14 and 28 days after COMIRNATY vaccination in the immediate and control groups.
- Differences in immune response at the different time points by both immunoassay (antibodies against SARS-CoV-2 spike protein) and NAV tests.
- Percentage of patients reaching a FRNT50 GMTs >1000 at 14 and 28 days (or the figure having best evidence in the literature at the time of analysis)
- Maintaining a FRNT50 GMTs >1000 (or the figure having best evidence in the literature at the time of analysis) at 3, 6 and 12 months.

A full description of statistical analysis will be made in the SAP.

Safety Analysis:

All clinical safety data will be listed by participant, time form vaccination dose and treatment arm. Continuous variables will be summarized using sample size (N), mean, standard deviation, median, minimum, and maximum. Frequency counts will be reported for categorical data.

A Data and Safety Monitoring Committee (DSMC) will review data for decisions on arms incorporation and withdrawal and will ensure that the appropriate oversight and monitoring in conducting the clinical trial. Interim analysis could be planned as necessary in each sub-protocol.

Trial Duration:

First analysis (completed 14 day) will be available about 5 weeks after first patient inclusion.

Each patient will be followed for 1 year.

End of Study Definition:

The end of study is considered as the last visit for the last participant in the study

1.2. Flowchart.

Group 1

Visit number		**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**
Procedure	**Screening**	**Randomization/ Boost**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**End of study**
Day ± window	0 to 7 days before boost vaccination	0	7±2	14 ±3	28±3	90±10	180±10	360±14
Screening for eligibility								
Eligibility check	X							
Informed consent	X							
PCR		X						
Baseline procedures								
Randomization		X						
Record demographic information	X							
Pregnancy test in urine	X							
Record medical history	X							
Record medication history	X	X						X
Complete physical examination	X							
Measure vital signs[1]	X			X	X			
Lab determinations	X[2]	X[2]			X			
Vaccination		X						
Endpoint assessment								
Assess acute reactions for at least 15 minutes after study intervention administration		X						
Solicited local and systemic adverse events (AEs) for 7 days after vaccine . Via E-diary		X						
Unsolicited local and systemic adverse events (AEs) for 28 days after vaccine . Via E-diary		X[3]						
Unsolicited SAEs		X						
Medically-attended adverse events (MAAEs)		X						
Immune response evaluation		X	X	X	X	X	X	X

1. Temperature, blood pressure and heart rate
2. Lab determination could be performed either at screening vist or randomization3. Two days per week

Group 2 (if crossing over is decided) *

Visit number		**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**
Procedure	**Screening**	**Randomization**	**Visit 2**	**Visit 3**	**Visit 4 – Boost dose**	**Visit 5**	**Visit 6**	**End of study**
Day ± window	0 to 7 days before boost vaccination	0	7±2	14 ±3	28±3	56±10	180±10	360±14
Screening for eligibility								
Eligibility check	X							
Informed consent	X							
PCR		X			X			
Baseline procedures								
Randomization		X						
Record demographic information	X							
Record medical history	X							
Record medication history	X	X						X
Complete physical examination	X				X			
Pregnancy test in urine	X				X			
Measure vital signs[1]	X	X						X
Lab determinations	X[2]	X[2]			X	X		
Vaccination					X			
Endpoint assessment								
Assess acute reactions for at least 15 minutes after study intervention administration					X			
Solicited local and systemic adverse events (AEs) for 7 days after vaccine. Via E-diary					X (7 days)			
Unsolicited local and systemic adverse events (AEs) for 28 days after vaccine . Via E-diary					X[3]			
Unsolicited SAEs		X						
Medically-attended adverse events (MAAEs)		X						
Immune response evaluation		X	X	X	X	X	X	X

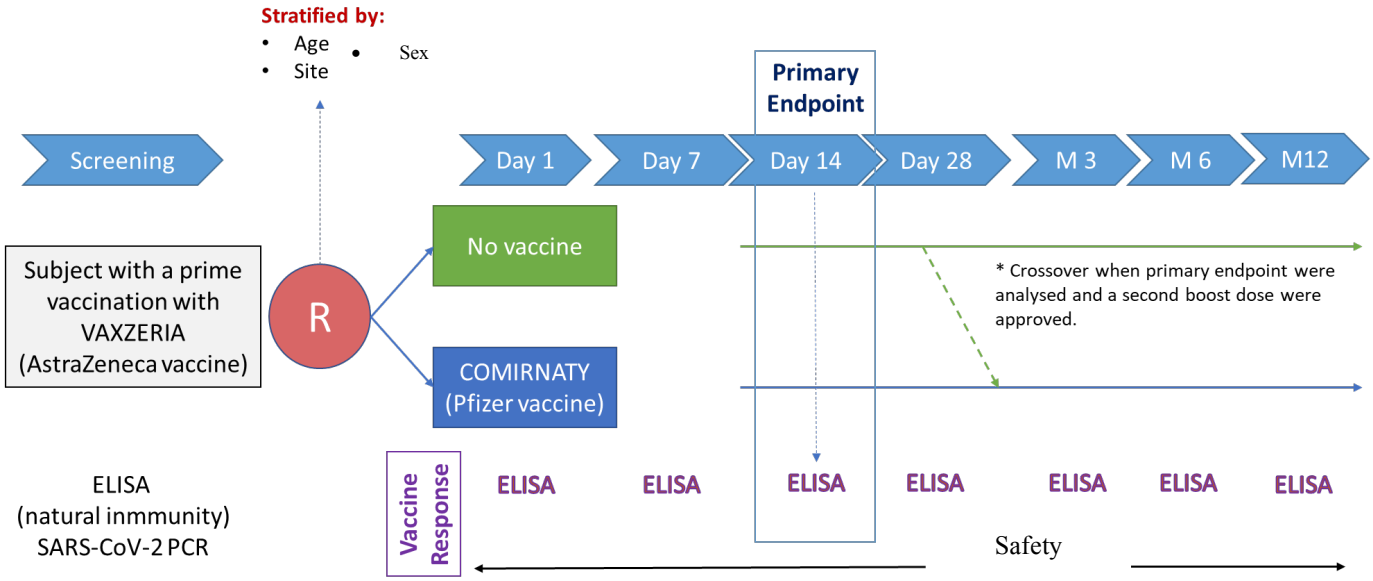
Note: If subjects in Group 2 are not crossed over, the visits will be the same as the groups 1.

1. Temperature, blood pressure and heart rate
2. Lab determination could be performed either at screening vist or randomization
3. Two days per week

Participants with COVID19-like signs and symptoms

Procedure	**Symptoms**	**Confirmation**	**Early Follow up**	**Late Follow up**
Day ± window	Day 1-2	Day 2-5	Day 6 to 28	Day 29±7
Site to contact participant if COVID19 signs or symptoms are recorded in the e-Diary	X			
Case evaluation	X			
Nasal swab sample		X		
Humoral immunity		X		X
Biomarker RNAseq		X		X
Vital signs, including pulse oximetry		X		X
Symptoms of infection, including highest body temperature over the last 24 hours measured by participant		X		X

1.3. Trial schematic



Exclusion Criteria

Patient with previous infection by SARS-CoV-2

Primary endpoints:

- Humoral immune response against SARS-Cov-2, 14 days after a boosted vaccination with COMIRNATY in subjects that received a prime vaccination with VAXZERIA

2. Introduction

2.1. Study Rationale and Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causal agent of coronavirus disease 2019 (COVID-19) outbreak. Declared as a pandemic on March 11, 2019 by the World Health Organisation, COVID-19 outbreak is an evolving global health emergency. A huge effort for prevention and treatment of COVID-19 has since been devoted globally. As of February 16, 2021, almost 5000 trials related with COVID-19 have been registered.

Vaccination against COVID-19 started on 27 December 2020 across the European Union. Four vaccines have been so far given conditional marketing authorisation by the European Commission: mRNA vaccine BNT162b2 (BioNTech and Pfizer - COMIRNATY), mRNA vaccine CX-024414 (Moderna), adenovirus vaccine ChAdOx1-S (AstraZeneca - VAXZEVRIA) and adenovirus vaccine Ad26.Cov2.S (Janssen-Cilag International NV). These vaccines are indicated for active immunisation to prevent COVID-19 caused by SARS-CoV-2 in individuals 16 or 18 years of age and older. Most coronavirus vaccines are given as two injections: an initial 'prime' dose followed by a 'second' to stimulate the immune system's memory cells and amplify the immune response (only Ad26.Cov2.S vaccine has been approved as a single-dose). However, their safety and effectiveness using them as heterologous vaccination regimens have not yet evaluated.

Due this pandemic situation, the ability to mix and match vaccines could make vaccination programmes more flexible, speeding up the process and reducing the impact of any supply-chain disruptions. This concept has been also used in other vaccines, as Ebola one. A heterologous prime-vaccination combination of Ebola Virus Vaccine was evaluated in healthy adult subjects in a clinical trial, concluding that the standard and accelerated heterologous prime-boost regimens were well-tolerated and elicited potent cellular and humoral immunogenicity. Therefore, this heterologous prime-boost regimen was approved last year by European regulators to protect against Ebola.

Also, induction of immune response by combining vaccines using different platforms could be stronger. It has been shown that some vaccines produce a better antibodies response while others best stimulate cellular response.

In the case of vaccine against SARS-CoV-2 infection, some trials are ongoing using heterologous prime boost regimen for the prevention of COVID19: 1) NCT04760730 trial use both adenovirus vector vaccines (VAXZEVRIA and rAd26-S), 2) NCT04776317 trial use Chimpanzee Adenovirus serotype 68 (ChAd) and self-amplifying mRNA (SAM) vectors in healthy adult subjects including older adult subjects, and 3) NCT04684446 trial evaluate the safety and immunogenicity of VAXZEVRIA given in combination with (either before or after) rAd26-S.

CombiVacS is the first clinical trial evaluating the immunogenicity and safety of second vaccination with a mRNA COVID19 vaccine (COMIRNATY – Pfizer) in subject having received prime vaccination with a viral vector COVID19 vaccine (VAXZEVRIA – AstraZeneca).

2.2. Benefit/Risk Assessment

While the efficacy and safety profile of the approved COVID-19 vaccines are subject to investigation when used as heterologous vaccination regimen within this protocol, the only contraindication to COVID-19 vaccines has been determined as hypersensitivity to the active substance or to any of the excipients within the vaccine product. Therefore, participants volunteering for this trial are not considered to be at additional risk related to the administration of the COVID-19 vaccine.

Participants in this study are exposed to some general risks. The participants may be subjected to additional blood sampling without representing any relevant harm or discomfort. Collection of other samples, like oropharyngeal or nasal swabs, or urine, are considered not to cause any significant harm.

Participants' identities will be protected, and personal health information (PHI) held securely. No directly identifiable data will be stored in the clinical trial database, and the participant lists will be stored separately, secured, at the local study sites. The risk that unauthorized persons will see the participant's PHI will be kept at a minimum, and measures will be taken to keep PHI confidential and in accordance with the legal requirements.

3. Objectives and Endpoints

The hypothesis of this trial is that in patients having received a prime dose with VAXZEVRIA immunogenicity after a boosted dose with COMINARTY is better than if no boosted vaccination is done.

Table 3.1: Objectives and corresponding endpoints

Objectives	Endpoints
Primary Immunogenicity Objective	
To assess the humoral immune response against SARS-CoV-2, 14 days after a vaccination with COMIRNATY in subjects that received a previous single dose of VAXZEVRIA, as compared with no dosing.	- Serological response to vaccination (antibodies against SARS-CoV-2 spike protein) as measured by immunoassay, 14 days after the boost dose.
Exploratory objective	
To evaluate the relationship between the immune response measured as NAV (Neutralizing antibodies) and antibodies against SARS-CoV-2 spike protein measured by immunoassay.	- NAV (Neutralizing antibodies) and immunoassay (enzyme-linked immunosorbent assay).
To assess the cellular immune response against SARS-CoV-2, 14 and 28 days after a vaccination with COMIRNATY in subjects that received a previous single dose of VAXZEVRIA, as compared with no dosing.	- Cellular response to vaccination (inflammatory cytokine production IFNg and IL2 against SARS-CoV-2 spike protein) as measured by ELISA immunoassay, basal, 14 and 28 days after the boost dose.
Secondary immunogenicity objectives	
To assess the humoral immune response against SARS-CoV-2, 28 days after a vaccination with COMIRNATY in subjects that received a previous single dose of VAXZEVRIA.	- Serological response to vaccination (antibodies against SARS-CoV-2 spike protein) as measured by immunoassay, 28 days after the boost dose. - SARS-CoV-2 neutralization as measured by virus neutralization assay, 14 and 28 days after the boost dose.
To assess the long-term (up to 1 year) humoral immune response against SARS-CoV-2 of a dose of COMIRNATY in subjects that received a previous single dose of VAXZEVRIA.	-Serological response to vaccination (antibodies against SARS-CoV-2 spike protein) as measured by immunoassay at 3, 6 and 12months after the boost dose. - SARS-CoV-2 neutralization as measured by virus neutralization assay at 3, 6 and 12 months after the boost dose.
To assess the humoral immune response against viral variants of SARS-CoV-2, 14 and 28 days after a dose of COMIRNATY in subjects that received a previous single dose of VAXZEVRIA.	-Serological response to vaccination (antibodies against SARS-CoV-2 spike protein) as measured by immunoassay at 3, 6 and 12months after the boost dose. - SARS-CoV-2 neutralization as measured by virus neutralization assay at 3, 6 and 12 months after the boost dose.
Secondary efficacy objective	
To assess the occurrence of symptomatic molecularly confirmed COVID-19 and severity of COVID-19 signs and symptoms after the administration of a dose of COMIRNATY in subjects that received a prior single dose of VAXZEVRIA.	- The number of participants with molecularly confirmed COVID-19. - Presence and severity of COVID-19 signs and symptoms as measured by Symptoms of Infection with Coronavirus-19 (SIC).
Safety	
To evaluate the safety of a dose of COMIRNATY in subjects that received a previous single dose of VAXZEVRIA.	- Solicited local and systemic adverse events (AEs) for 7 days after vaccine - Unsolicited local and systemic adverse events (AEs) for 28 days after vaccine (Two days per week)

	- Serious adverse events (SAEs) throughout the study (from randomization until end of the study). - Medically-attended adverse events (MAAEs) from the day of vaccination until 6 months after the last vaccination - Lab parameters: biochemistry and haematology at 1 month visit.

4. Study Design

4.1. Overall Design

This is a randomized, non-blinded, controlled, adaptive, multicenter, Phase II study in subjects aged more than 18 years and in good health or stable clinical situation that have received a previous single dose of VAXZEVRIA.

This is a phase 2 adaptive trial developed for evaluating the immunogenicity of a dose of COMIRNATY after a previous single dose with VAXZEVRIA. A stratification will be made for the following factors: site of vaccination, sex and age. This protocol allows to test the immunogenicity and safety of a heterologous vaccination strategy after a single dose of VAXZEVRIA.

Subjects will be randomized to immediately receive or not dose of COMIRNATY in a proportion of 2:1.

If primary analysis at 14 days confirms the starting hypothesis, subjects randomized to no vaccination will be considered for administration of one dose of COMINARTY at day 28th according to Public Health Department of the Ministry of Health recommendations on heterologous vaccination. If vaccinated, subjects will be followed at the time points defined in the flowchart.

In case the primary analysis does not confirm the starting hypothesis, subject will be followed without administration of COMINARTY.

In case Public Health Department gave instructions to give the second dose of VAXZEVRIA to those subjects having received the first dose, subject will be followed as indicated in flowchart of Group 2.

Other heterologous vaccination strategies could be incorporated if deemed necessary for public health reasons. This could include the use of different boost strategies including those already marketed vaccines for comparative assessment of their safety and efficacy on SARS-CoV-2 and its variants.

The different intervention arms planned (and its corresponding control arm) can be carried-out in different trial sites, thus not all trial sites must participate in all arms.

Selection sample:

A multistage cluster sampling stratified by age groups will be carried out to obtain the number of subjects to be selected from the list of 400,000 subjects registered by Spanish Government as having received only the first dose of AZ vaccine. The first stage will be the autonomous community, the second the postcode and the third the hospital centre. Subjects will be selected in this last stage by systematic sampling stratified by age group (18-49, 50-59, and 60 and over years).

Randomisation and blinding:

- Randomization will be centralized, and each participant will be randomized stratified by age and centre. Randomization will be in a proportion of 2:1 (COMIRNATY:No vaccination)
- This is an open-label trial.

4.2. Scientific Rationale for Study Design

There is an urgent need to take decisions about how to proceed in those subjects having received a first dose of VAXZEVRIA during the Covid-19 vaccination campaign in Spain, after the decision taken by public health authorities put on hold the second dose of Vaxzevria. In addition, the ability to mix and match vaccines could make vaccination programmes more flexible, speeding up the process and reducing the impact of any supply-chain disruptions. At this moment no information exists on the safety and immunogenicity response for heterologous vaccination schemes. Therefore, a pragmatic trial is proposed to obtain enough information for public rapid decision making and to allow long-term evaluation on the safety and immunogenicity response after the boost dose of a mRNA vaccine (COMINARTY) in these subjects.

4.3. Justification for Dose

The dose of COMINARTY to be used for vaccination is the approved dose of this vaccine.

4.4. End of Study Definition

Participant Completion

A participant is considered to have completed the study if he/she has completed all study 1 year visits.

Study completion.

The study is considered complete when the last participant entering the trial has completed his/her last visit.

5. Study Population

Adult subjects (18 years old or higher) in good health or stable clinical situation volunteering to participate in the trial will be recruited after giving informed consent.

Selection criteria for inclusion of subjects in the trial will be maintained as pragmatic as possible.

5.1. Inclusion Criteria

Each potential participant must satisfy all of the following criteria to be enrolled in the study:

1. Adult subjects (18 years old) having received a prime VAXZEVRIA vaccination between 8 and 12 weeks before the screening visit.
2. Participants must provide consent indicating that he or she understands the purpose, procedures and potential risks and benefits of the study, and is willing to participate in the study.
3. Subjects in good health or stable clinical situation.
4. Participant is willing and able to adhere to the procedures specified in this protocol.

5.2. Exclusion criteria

Any potential participant who meets any of the following criteria will be excluded from participating in the study:

1. Participant has a clinically significant acute illness (this does not include minor illnesses such as diarrhea or mild upper respiratory tract infection) or temperature ≥38.0ºC within 24 hours prior to the planned dose of study vaccine.
2. Participant has a known or suspected allergy or history of anaphylaxis or other serious adverse reactions to COMIRNATY excipients.
3. Subjects with any contraindication to the administration of COMIRNATY, included pregnancy.
4. Subjects with prior documented COVID19 since VAXZEVRIA vaccination.
5. Subjects have symptoms or signs compatible with COVID19.
6. Subjects participating in a clinical trial in the last three months.
7. Any condition or situation precluding or interfering the compliance with the protocol.

6. Study Interventions and Concomitant Therapy

Study intervention is defined as heterologous boosted strategy based on approved SARS-CoV-2 vaccine intended to be administered to a study participant according to this protocol.

6.1. Study Intervention(s) Administered

6.1.1. Intervention arms:

Intervention arms (Group 1) will be the arm vaccinated with COMIRNATY after a previous vaccination with VAXZEVRIA between 8 and 12 weeks before the screening visit.

6.1.2. Control arm:

The control arm (Group 2) will have no vaccine administration. I If primary analysis at 14 days confirms the starting hypothesis, subjects randomized to no vaccination will be considered for administration of one dose of COMINARTY at day 28th according to Public Health Department of the Ministry of Health recommendations on heterologous vaccination. If vaccinated, subjects will be followed at the time points defined in the flowchart (Group 2).

6.2. Preparation, Handling, Storage, and Accountability

All study vaccine must be stored in a secured location with no access for unauthorized personnel and at controlled temperatures as indicated on the commercial labels and manufacturer instruction. If study vaccine is exposed to temperatures outside the specified temperature range, all relevant data will be sent to the manufacturer and sponsor to determine if the affected supplies can be used or will be replaced. The affected study vaccine must be quarantined and not used until further instruction from the sponsor is received.

Instructions for the preparation, handling and storage will be those described in the Product Information Sheet of COMIRNATY approved by AEMPS.

The investigator is responsible for ensuring that all study vaccine received at the site is inventoried and accounted for throughout the study. The study vaccine administered to the participant must be documented on the vaccine accountability form. All study vaccine will be stored and disposed of according to the manufacturer's instructions.

6.3. Measures to Minimize Bias: Randomization

This is an open-label clinical trial.

The randomization list will be generated using software SAS 9 for Windows. The randomization list will be imported into the REDCap program so the researchers can randomize candidate subjects using an easier-to-use interface. Subjects that meet the selection criteria will be randomized in a 2:1 ratio between the two treatment groups (Intervention arm: administration of a COMINARTY boosted dose vs Control arm: no administration of boosted dose), stratifying by center, gender and age (18-49 years, 50-59 years and ≥60 years), in order to achieve balanced randomization in the two treatment groups. The assignment of treatment to each subject will be centralized, keeping the sequence hidden.

6.4. Study Intervention Compliance

This study will be conducted within hospitals. Treatment is administered at site and participants will receive study intervention directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. Deviation(s) from the prescribed dosage regimen should be recorded. Intervention start and stop dates, will also be recorded.

6.5. Concomitant Therapy

Any medication or vaccine (including over the counter or prescription medicines and/or recreational drugs) at the time of enrolment or received during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

7. Discontinuation of Study Intervention and Participant Discontinuation/Withdrawal

7.1. Discontinuation of Study Intervention

This is not applicable. Intervention is a unique dose of COMIRNATY in the randomization visit.

7.2. Participant Discontinuation/Withdrawal from the Study

A participant may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, or compliance reasons. This is expected to be uncommon.

When a participant withdraws before study completion, the reason for withdrawal is to be documented in the eCRF and in the source document. If the reason for withdrawal from the study is withdrawal of consent, then no additional assessments are allowed. The participant will be permanently discontinued from the study intervention and the study at that time.

If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the study site records.

7.3. Lost to Follow-Up

To reduce the chances of a participant being deemed lost to follow-up, prior to randomization attempts should be made to obtain contact information from each participant, eg, home, work, and mobile telephone numbers and email addresses for both the participant as well as appropriate family members.

A participant will be considered lost to follow-up if he/she is unreachable for the study site per telephone directly to the patient or relatives (for at least in two occasions), or by written correspondence. A participant cannot be deemed lost to follow-up until all reasonable efforts made by the study-site personnel to contact the participant are deemed futile.

8. Study Assessments and Procedures

General instructions
Study procedures and their timing are summarized in Flow Chart (section 1.2). Protocol waivers or exemptions are not allowed.

Safety concerns should be discussed with the sponsor or sponsor representative immediately upon notice, to determine if the participant should continue or discontinue the study intervention.
Adherence to the study design requirements, including those specified in the flow chart, is essential and required for study conduct.

Screening visit
Screening for eligible participants will be performed within ≤7 days before randomization. The inclusion and exclusion criteria for enrolling participants in this study are described in section 5. If there is a question about these criteria, the investigator must consult with the sponsor (instituto de Salud Carlos III) representative and resolve any issues before enrolling a participant in the study. Waivers are not allowed.
The study will be explained to the participant and informed consent will be obtained prior to any study procedure.
After screening and obtaining informed consent, the following procedures will be performed:

- Record demographic information.
- Record medical history.
- Record concomitant medications and vaccinations if taken within 6 months prior to enrolment in this trial.
- Perform a complete physical examination, including height and weight.
- Measure vital signs (body temperature, pulse, blood pressure).
- Pregnancy test

Visit 1. Randomization

- PCR
- Review medical history
- Review medication history
- Measure vital signs (body temperature, pulse, blood pressure).
- Blood extraction for immune response evaluation.
- Blood extraction for lab safety determinations.
- Randomization.
- Pregnancy test

Vaccination arm

- Review criteria for cancellation of vaccination.
- Administer the trial vaccine dose according to the subject's assignment and administration guidance.
- Observe the subject on site for at least 15 minutes following vaccination for safety monitoring. At the end of the observation period, record the occurrence of any AEs following trial vaccination.

Visit 2 (Day 7±2)

- Inquire about AEs and general well-being of the participant since the administration of vaccine dose. Record any AEs, 'solicited' and 'unsolicited' AEs, SAEs and MAAE.
- Review medication history
- Measure vital signs (body temperature, pulse, blood pressure).
- Blood extraction for immune response evaluation

Visit 3 (Day 14±3)

- Inquire about AEs and general well-being of the participant since the administration of vaccine dose. Record any AEs, 'unsolicited' AEs, SAEs and MAAE.
- Review medication history
- Measure vital signs (body temperature, pulse, blood pressure).
- Blood extraction for immune response evaluation

Visit 4 (Day 28±3)

- Inquire about AEs and general well-being of the participant since the administration of vaccine dose. Record any AEs, 'unsolicited' AEs, SAEs and MAAE.
- Review medication history
- Measure vital signs (body temperature, pulse, blood pressure).
- Blood extraction for immune response evaluation
- Lab determination

Control arm (Group 2)

- Review criteria for cancellation of vaccination.
- PCR
- Administer the trial vaccine dose according to the subject's assignment and administration guidance.
- Observe the subject on site for at least 15 minutes following vaccination for safety monitoring. At the end of the observation period, record the occurrence of any AEs following trial vaccination.

Visit 5 (Day 90±10 or 56±3):

- Inquire about AEs and general well-being of the participant since the administration of vaccine dose. Record any AEs, 'solicited' and 'unsolicited' AEs, SAEs and MAAE.
- Review medication history
- Measure vital signs (body temperature, pulse, blood pressure).
- Blood extraction for immune response evaluation
- Lab determination at 56±3 for group 2 in case of COMIRNATY vaccination

Visit 6 (Day 180±10):

- Inquire about AEs and general well-being of the participant since the administration of vaccine dose. Record any AEs, 'unsolicited' AEs, SAEs and MAAE.
- Review medication history
- Measure vital signs (body temperature, pulse, blood pressure).
- Blood extraction for immune response evaluation

Visit 7 (Day 360±10):

- Inquire about AEs and general well-being of the participant since the administration of vaccine dose. Record any AEs, 'unsolicited' AEs, SAEs and MAAE.
- Review medication history
- Measure vital signs (body temperature, pulse, blood pressure).
- Blood extraction for immune response evaluation

8.1. Immunogenicity Assessments

Planned time points for all immunogenecity and clinical efficacy assessments are provided in the the Flow-Chart (section 1.2 Flowchart). Details of the efficacy assessments are provided below.

All samples for immunogenicity test will be sent to "Centro Nacional de Microbiología" for centralized analysis. After all planned test in this protocol are finalized, all samples will be stored in the ISCIII during 15 years. Detailed sampling shipping instructions will be sent to participating sites from the laboratory.

8.1.1. Primary immunogenicity assessment.

The primary immunogenicity assessment will take place 14 days after the boost dose by serological response to vaccination as measured by immunoassay. All determinations will be performed at central level in "Centro Nacional de Microbiología".

8.1.2. Secondary immunogenicity assesments.

Secondary immunogenicity assessments will include:

- Serological response to vaccination as measured by immunoassay, 28 days after the COMIRNATY dose.

- Serological response to vaccination as measured by immunoassay at 3, 6 and 12months after the COMIRNATY dose.
- Neutralizing antibody titers at 14 and 28 days and 3, 6 and 12 months after the COMIRNATY dose.

Exploratory assessment includes:

- Cellular response to vaccination (inflammatory cytokine production IFNg and IL2 against SARS-CoV-2 spike protein) as measured by ELISA immunoassay, basal, 14 and 28 days after the boost dose.

8.2. Efficacy assessments

Occurrence of confirmed COVID-19 is defined as the presence of a documented positive SARS-CoV-2 test performed within a healthcare facility. Classification of severity will be based on the highest degree of severity during the observation period.

In general, adults with SARS-CoV-2 infection can be grouped into the following severity of illness categories. However, the criteria for each category may overlap or vary across clinical guidelines and clinical trials, and a patient's clinical status may change over time.

- Asymptomatic or Presymptomatic Infection: Individuals who test positive for SARS-CoV-2 using a virologic test (i.e., a nucleic acid amplification test or an antigen test) but who have no symptoms that are consistent with COVID-19.
- Mild Illness: Individuals who have any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but who do not have shortness of breath, dyspnea, or abnormal chest imaging.
- Moderate Illness: Individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have saturation of oxygen (SpO2) ≥94% on room air at sea level.
- Severe Illness: individuals hospitalized needing oxygen by NIV or high flow, re-breather mask.
- Critical Illness: Individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction.

8.3. Safety Assessments

The definitions of an AE and an SAE and MAAE can be found in Table 8.4.1.

Planned time points for all safety assessments are provided in the Flow-Chart. Unscheduled clinical laboratory measurements may be obtained at any time during the study to assess any perceived safety issues.

A clinical assessment, including medical history, will be performed on all participants at his/her first visit to establish a baseline. Significant medical history and observations from any physical examination, if performed, will be documented in the eCRF.

Acute reactions within the first 15 minutes will be assessed and documented in the eCRF.

AEs will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible to pursue and obtain adequate information both to determine the outcome and to assess whether the event meets the criteria for classification as an SAE or caused the participant to discontinue the study intervention.

AEs will be documented in the eCRF for each visit as determined in the Flowchart.

8.4. Adverse Events (AEs), Serious Adverse Events (SAEs), and Other Safety Reporting

The definitions of the EU Directive 2001/20/EC Article 2 based on the principles of GCP and EU guidance ENTR/CT 3 apply to this trial protocol, when required local requirements should also apply.

Table 8.4.1: Definitions of Adverse Events

TERM	DEFINITION
Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical trial participant, which does not necessarily have a causal relationship with the research procedures or the investigational medicinal product (IMP).
Adverse Reaction (AR)	Any untoward and unintended responses to an IMP related to any dose administered.
Serious Adverse Event or Reaction (SAE/SAR)*	Any AE/AR that, at any dose, results in: - death; - a life-threatening AE; - hospitalization or prolongation of existing hospitalization; - a persistent or significant disability or incapacity; - a congenital anomaly/birth defect; - an "important medical event"
Suspected Unexpected Serious Adverse Reaction (SUSAR)	An unexpected adverse reaction is an AR of which the nature, outcome, frequency or severity is not consistent with the applicable Reference Safety Information (RSI): SmPC or Investigator's Brochure (IB).
Medically Attended Adverse Events (MAAEs)	Adverse events with medically-attended visits that were not routine visits for physical examination or vaccination, such as visits for hospitalization, an emergency room visit, or an otherwise unscheduled visit to or from medical personnel (medical doctor) for any reason
New fact**	Any safety data that could modify significantly the evaluation of the benefit/risk ratio of the IMP or the clinical trial, likely to affect the safety of participants or that could modify the IMP administration, the trial documentation or the conduct of the trial, or to suspend, interrupt or modify the protocol or similar trials.

** EXCEPTIONS: the following events are not considered as SAE requiring immediate reporting to the sponsor:*
-the participant is formally admitted to a hospital for medical reasons with no seriousness criterion and does not require overnight hospitalization
-elective or previously scheduled surgery or medical treatment;
-hospitalization for social or administrative reasons;
-pre-existing diseases or present conditions detected prior to start of study drug administration and which do not worsen.

***Example: a SAE which could be associated with the trial procedures and which could modify the conduct of the trial, a significant hazard to the subject population such as lack of efficacy of an IMP used for the treatment of a life-threatening disease, recommendations of the data safety monitoring board, if any, where relevant for the safety of subjects.*

8.4.1. Time period and frequency for collecting and reporting AE and SAE information

- All AEs and SAEs will be collected from the signing of the informed consent form (ICF) until the follow-up visit at the time points specified in the Flowchart.

- All AEs and SAEs that are related to investigational products will be recorded from the time of ICF signing to end of study or early study discontinuation.

- Any clinically relevant event that occurs between signing of the ICF and the first vaccination will be collected and reported as pre-existing medical condition.

- All information on AEs (non-serious and serious) and pregnancies should be recorded on the appropriate eCRF (eCRF completion will be detailed into eCRF user manual).

- All AEs will be monitored until resolution or clinical stabilization.

- SAE information will be transmitted using a SAE form. The form should be reviewed and signed by an investigator and must be transmitted within 24 hours from initiation of SAE.

- All events that meet the definition of a SAE will be reported as SAEs, regardless of whether they are protocol-specific assessments.

8.4.2. Method of detecting AEs and SAEs

- Care will be taken to not introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

8.4.3. Assessment of AEs and SAEs

Seriousness
For any adverse event, the investigator must determine whether the event meets one or more of the seriousness criteria described in Table 8.

Table 8. AE severity scale

Grade 1 (Mild)	Mild or transient discomfort, without limitation of normal daily activities; no medical intervention or corrective treatment required.
Grade 2 (Moderate)	Mild to moderate limitation of normal daily activities; minimal medical intervention or corrective treatment required.
Grade 3 (Severe)	Marked limitation of normal daily activities; medical intervention and corrective treatment required, possible hospitalization.
Grade 4 (Life-threatening)	Severe limitation of normal daily activities; medical intervention and corrective treatment required, almost always in a hospital setting.

Causality
The investigator must assess the causality of all AEs/SAEs in relation to IMP, research procedures, and concomitant medications, using the following guidelines:

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate aetiology has been established.

All AEs/SAEs for which the investigator or the sponsor considers a causal relationship to be a reasonable possibility are considered suspected SARs.
Assessment on expectedness is usually done by the sponsor.

8.5. Regulatory reporting requirements for serious adverse events

The sponsor assumes responsibility of reporting AE s and SAEs to the corresponding regulatory authorities. At the same time, the sponsor must report to the investigator all suspected unexpected serious adverse reactions, known as SUSARs. SUSARs must be reported as well to the corresponding IEC/IRB unless otherwise indicated by institution.

9. Statistical Considerations

The statistical analysis plan (SAP) will be finalized prior to database lock and it will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints including primary and key secondary endpoints.

9.1. Statistical Hypotheses

The efficacy hipotesis is:
Ho: G(Y1) / G(Y2) = 1 versus H1: G(Y1) / G(Y2) > 1
With definition:
G(Y1) is the titer of antibodies binding to the SARS-CoV-2 S protein at 14th day after a dose of COMIRNATY in subjects that received a prior single dose of VAXZEVRIA.
G(Y2) is the titer of antibodies binding to the SARS-CoV-2 S protein at 14th day in subjects no receiving the COMIRNATY dose at randomization.
The antibodies binding to the SARS-CoV-2 S protein will be measured as lognormal distribution.

9.2. Analysis Sets

For the purposes of analysis, the following analysis sets are defined:

Participant Analysis Set	Description
Full analysis set	• All participants having received at least one dose of study intervention and who have at least one efficacy evaluation after baseline. Participants will be included in the analyses according to the planned randomized intervention.
Safety analysis set	• All participants who are exposed to a study intervention. Participants will be analysed according to the intervention they actually received.

The full analysis set is used to analyse endpoints related to the efficacy objectives and the safety analysis set is used to analyse the endpoints and assessments related to safety.

The following data points sets are defined:

Defined Data Points Sets	Description
DPS1	• All observed data will be included in the analysis set.
DPS2	• For participants who discontinue study intervention and/or receive rescue therapy, post-discontinuation or post-rescue observations will not be included.

Full analysis set and DPS1 are used to estimate the primary endpoints and the secondary endpoints for secondary core objective.
Full analysis set and DPS2 are used to for sensitivity analyses.
Safety analysis set and DPS1 are used to present safety data.

9.3. Statistical Analyses

9.3.1. General considerations

All analyses will be carried out using the statistical software SAS, version 9.4 of the SAS system for Windows.

All continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation (SD), 1st quartile, median, 3rd quartile, maximum and minimum. Absolute and relative frequencies (based on the non-missing sample size) of observed levels will be reported for all categorical variables.

All continuous variables will be tested for normality hypothesis assumption with the kolmogorov-Smirnov test. The antibodies binding to the SARS-CoV-2 S protein will be measured as lognormal distribution to obtain more nearly symmetric distribution.

The number of participants and the flowchart of the study will be presented. The period of enrolment and the total number of participants screened to be included in the study will be presented. The number of ineligible participants and the total number of randomized participants will be presented. The number of participants who never receive the study intervention will also be presented by treatment group and those who were included in the full analysis set and the safety analysis set will be presented.

Baseline is considered as the day 1 visit and the baseline data is data collected just before intervention at day 1. Participants with missing baseline value will be excluded from the analysis.

9.3.2. Primary endpoint(s) analysis

The immune response measured by immunoassay 14 days after randomisation between the two arms will be tested by one-tailed t-test assuming symmetric distribution of lognormal transformation. Geometric means will be calculated as the mean of the assay results after making the logarithm transformation and then exponentiating the mean to express results on the original scale. One-sided 95% CIs will be obtained by taking natural log transforms of concentrations/titers, calculating the 95% CI with reference to the t-distribution, and then exponentiating the confidence limits. In other case, Mann-Whitney-Wilcoxon test will be used.

9.3.3. Secondary endpoint(s) analysis

Secondary immunogenicity endpoints (serological response and neutralizing antibodies at each time point) between the two arms will be tested by one-tailed t-test assuming symmetric distribution of lognormal transformation. Geometric means will be calculated as the mean of the assay results after making the logarithm transformation and then exponentiating the mean to express results on the original scale. One-sided 95% CIs will be obtained by taking natural log transforms of concentrations/titers, calculating the 95% CI with reference to the t-distribution, and then exponentiating the confidence limits. In other case, Mann-Whitney-Wilcoxon test will be used.

For secondary efficacy objectives, the absolute and relative frequencies of individuals with molecularly confirmed COVID-19 and SIC will be compared through the two-tailed chi-square test and alpha error equal to 0.05.

9.3.4. Exploratory/subgroup analysis

No subgroup analysis will be carried out.

9.3.5. Safety analyses

Safety endpoints include serious adverse events (SAEs) leading to study treatment discontinuation and death. SAEs will be coded using the current version of the Medical Dictionary for Regulatory Activities (MedDRA). SAEs leading to study treatment discontinuation and death will be summarized with absolute and relative frequencies (%) with 95% confidence intervals (CIs). More details on the statistical analysis will be provided in the SAP.

9.4. Interim Analysis

No formal interim analysis for efficacy will be done. However, an independent data monitoring committee consisting of independent scientists not otherwise involved in the study has been appointed and will review the data regularly during the study for safety and scientific integrity and will make recommendations to the sponsor regarding the stopping of an intervention for harm or for futility. The frequency of the committee data review meetings and other aspects such as stopping rules will be detailed in a separate charter depending on the safety profile of the study intervention(s).

9.5. Sample Size Determination

Sample size calculation for a log-transformed outcome measure (Wolfe R, Carlin JB. Sample-size calculation for a log-transformed outcome measure. Control Clin Trials. 1999 Dec;20(6):547-54. doi: 10.1016/s0197-2456(99)00032-x. PMID: 10588295) has been performed to assess the humoral immune response against SARS-CoV-2 14 days after dose of COMIRNATY in subjects that received a prior single dose of VAXZERIA, as compared with no dosing. Sample size to obtain will be 600 participants divided in 400 in arm1 and 200 in arm2 (2:1 randomization ratio): we assume that: a) increase in antibodies titer would be at least x1.35 in subjects receiving the dose of COMIRNATY G(Y1) in relation with those not receiving it G(Y2); b) a coefficient of variation equal to 1.2 or 1.0 and similar between arms, c) a 80% statistical power and alpha equal to 0.01 for one side test (H1: G(Y1)/G(Y2) >1). See table below for different assumptions in coefficient of variation and G(Y1)/ G(Y2). The planned sample size is also considered appropriate to evaluate safety endpoints.

The sample size will be increased by 15% of the participants being non-evaluable.

Case	alfa	beta	2:1	Coef. Var	G(Y1)/G(Y2)	VAXZERIA group (n=)	COMIRNATY group (n=)
2	0.01	0.2	2	1.2	1.35	173.506	347.012
3	0.01	0.2	2	1.2	1.40	138.026	276.052
4	0.01	0.2	2	1.2	1.45	113.186	226.372
5	0.01	0.2	2	1.2	1.50	95.050	190.100
7	0.01	0.2	2	1.1	1.35	154.248	308.496
8	0.01	0.2	2	1.1	1.40	122.706	245.412
9	0.01	0.2	2	1.1	1.45	100.623	201.247
10	0.01	0.2	2	1.1	1.50	84.500	169.000
12	0.01	0.2	2	1.0	1.35	134.827	269.653
13	0.01	0.2	2	1.0	1.40	107.256	214.513
14	0.01	0.2	2	1.0	1.45	87.954	175.908
15	0.01	0.2	2	1.0	1.50	73.861	147.722

10. Supporting Documentation and Operational Considerations

10.1. Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1. Regulatory and ethical considerations

- This study will be conducted in accordance with the protocol and with the following:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) international ethical guidelines
    - Applicable ICH Good Clinical Practice (GCP) guidelines
    - Applicable laws and regulations

The protocol, protocol amendments, ICF, investigator's brochure and other relevant documents (eg, advertisements) must be submitted to a Research Ethics Committee (REC) and National Competent Authority (NCA) by the sponsor and reviewed and approved by the REC and NCA before the study is initiated.

Any amendments to the protocol will require REC and NCA approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

Protocols and any substantial amendments to the protocol will require health authority (NCA) approval prior to initiation except for changes necessary to eliminate an immediate hazard to study participants.

The sponsor will be responsible for the following:

- Providing written summaries of the status of the study to the REC/NCA annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the REC/NCA of SAEs or other significant safety findings as required by REC/NCA procedures
- Providing oversight of the conduct of the study at the sites and adherence to requirements of ICH guidelines, the REC/NCA, European Directive 2001/20/EC and European Regulation 536/2014 for clinical trials on medicinal products, and all other applicable local regulations

10.1.2. Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3. Informed Consent process

The investigator or his/her representative will explain the nature of the study, including the risks and benefits, to the participant or their legally authorized representative (LAR) and answer all questions regarding the study.

Participants must be informed that their participation is voluntary. Participants, their LAR or their designated representative (relative) will be required to give a statement of informed consent that meets the requirements, local regulations, ICH guidelines, privacy and data protection requirements, where applicable, and the REC or study centre.

The medical record must include a statement that informed consent was obtained before the participant was enrolled in the study, the nature of consent (participant, LAR, designed representative or independent doctor), and the date the consent was obtained. The authorized person obtaining the informed consent must also sign the ICF. In case of urgent oral consent is obtained it should be written in medical record.

Participants must be reconsented to the most current version of the ICF(s) during their participation in the study if relevant.

A copy of the ICF(s) must be provided to the participant, their legally authorized representative or their designated representative.

Participants who are rescreened are required to give a new consent.
The ICF will contain a separate section that addresses the secondary use of data and remaining samples for optional exploratory research. The investigator or authorized designee will explain to each participant the objectives of the exploratory research. Participants will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to document a participant's

agreement to allow any data and remaining specimens to be used for secondary exploratory research. Participants who decline to participate in this optional research will not provide this separate signature.

10.1.4. Data protection

Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred. Sponsor staff that require access to personal data will agree to keep confidentiality. Data relevant to fulfil the objectives of the study will be collected only.

The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent.

The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor and by inspectors from regulatory authorities. Study participants have the right to request access to their personal data and the right to request rectification of incorrect or incomplete data.

Exploratory research data will not be returned to participants or investigators, unless required by law or local regulations. Privacy and confidentiality of data generated in the future on stored samples will be protected by the same standards applicable to all other clinical data.

10.1.5. Data quality assurance

- All participant data relating to the study will be recorded on electronic CRFs. The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- Guidance on completion of eCRFs will be provided in the Trial Master File.
- The investigator must permit study-related monitoring, audits, REC/NCA review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy, including definition of study critical data items and processes (eg, risk-based initiatives in operations and quality such as risk management and mitigation strategies and analytical risk-based monitoring), methods, responsibilities, and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the monitoring plan.
- The sponsor or designee is responsible for the data management of this study, including quality checking of the data.
- The sponsor assumes accountability for actions delegated to other individuals (eg, contract research organizations).
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.6. Source documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Definition of what constitutes source data and its origin can be found in monitoring guidelines.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

10.1.7. Study and site start and closure

First Act of Recruitment

- The study start date is the date on which the clinical study will be open for recruitment of participants.
- The first act of recruitment is the informed consent and will be the study start date.

Study/Site Termination

- Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.
- The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.
- Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:
    - Failure of the investigator to comply with the protocol, the requirements of the REC/NCA or local health authorities, the sponsor's procedures, or GCP guidelines
    - Inadequate or no recruitment (evaluated after a reasonable amount of time) of participants by the investigator
    - Total number of participants included earlier than expected
- Reasons for early study termination by the sponsor may include but are not limited to:
    - Discontinuation of further study intervention development
    - Occurrence of AEs unknown to date in respect of their nature, severity and duration
    - Medical or ethical reasons affecting the continued performance of the trial, including
        - external evidence indicating efficacy or harm of any of the study interventions
        - a general lack of eligible patients due to vaccination or other reasons

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the RECs, the regulatory authorities (NCA), and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up.

10.1.8. Publication policy

The study will be registered in a public register (clinicaltrials.gov), in which trial results will be posted. The results of this study will be published in a suitable publication irrespective of findings. Results will also be presented at scientific meetings.

The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicentre studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements. Conflicts of interests will be disclosed. The contribution of ECRIN and its national partners will be fairly described in the acknowledgement section or as co-author, depending on the contribution in the trial design, planning and publication.
In line with the EU data sharing policy, individual patient-level data will be shared with the scientific community (either as anonymised or pseudonymised data sets), while maintaining the integrity and privacy of the trial participants and in compliance with the EU General Data Protection Regulation and local rules. Anonymized data will be made available using a data repository, including any programming code used to produce the trial results. Pseudonymized data will be made available in a secure environment, through a controlled access, upon request and evaluation of the requestee's ability and willingness to maintain the integrity and privacy of the trial participants. Further details of data sharing will be given in a separate data sharing plan.

10.2. Appendix 2: Clinical Laboratory Tests

10.2.1. Clinical Laboratory Tests

The tests detailed in table 10.2.1 will be performed by the local laboratory.

Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 10.2.1: Protocol-required Laboratory Tests

Laboratory Tests	**Parameters**
Haematology	
	Haemoglobin (g/dL)
	Platelet count (x 10^9/L).
	White blood cell count (x 10^9/L)
	Neutrophils (x 10^9/L)
	Lymphocytes (x 10^9/L) and its fractions.
Clinical chemistry	
	K, Potassium (mmol/L)
	Na, Sodium (mmol/L)
	Creatinine (µmol/L)
	Total bilirubin (µmol/L)
	Haemostasis study (at least INR)
	AST, Aspartate aminotransferase or SGOT, serum glutamic-oxaloacetic transaminase (U/L)
	ALT, Alanine aminotransferase or SGPT, Serum glutamic-pyruvic transaminase (U/L)
	Glucose (mmol/L)
Pregnancy testing	
	Pregnancy rapid test in urine

10.3. Appendix 3: Solicited AEs (applicable for injectable IMPs)

TYPE	EVENT	
Local AEs (injection or infusion site)	Discomfort Itching Redness Swelling (soft) Induration (hard) Blisters	
Systemic Clinical AEs following injections/infusions	Temperature Chills/night sweats Myalgia/flu-like general muscle aches Arthralgia Malaise (excess fatigue) Headache Nausea Vomiting Nasopharyngitis/Upper respiratory tract infection/cough/sinusitis Generalised rash Generalised itching	
Systemic Laboratory AEs	Creatinine ALT Alkaline phosphatase Total bilirubin INR Glucose Sodium Chloride	Haemoglobin Total White Cell Count Neutrophils Lymphocytes Platelets

10.4. Appendix 4: Abbreviations

AE	adverse event
ALT	alanine aminotransferase
AR	Adverse Reaction
AST	aspartate aminotransferase
CIOMS	Council for International Organizations of Medical Sciences
COVID-19	coronavirus-induced disease first described in 2019
CRF	case report file
DPS	Data Point Set
DSMC	Data Safety Monitoring Committee
DSUR	Development Safety Update Report
e.g.	example given
eCRF	electronic case report file
EMA	European Medicines Agency
GCP	Good Clinical Practice
IB	Investigator Brochure
ICF	informed consent form
ICH	International Council for Harmonization
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
INR	international normalized ratio
IRB	Institutional Review Board
K	potassium
LAR	Legally Authorized Representative
MedDRA	Medical Dictionary for Regulatory Activities
Na	sodium
NIV	non-invasive ventilation
O2	oxygen
PHI	personal health information
PI	principal investigator
pO2	partial pressure of oxygen
RNA	ribonucleic acid
RSI	Reference Safety Information
SAE	serious adverse event
SAP	statistical analysis plan
SAR	serious adverse reaction
SARS-CoV-2	SARS-coronavirus-2
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
SmPC	Summary of Product Characteristics
SpO2	oxygen saturation
SUSAR	suspected unexpected serious adverse reaction
TOC	table of contents
TSG	Trial Safety Group

10.5. Appendix 5: Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the table of contents (TOC).
Following amendments will be registered as indicated below:

Overall Rationale for the Amendment
Amendment 1: April 15, 2021
Protocol Version: 1.1
This amendment is considered to be nonsubstantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

Section # and Name	Description of Change	Brief Rationale
1.2 Flowchart	Procedures harmonization	Minor errors in the procedures of flowchart section
Apendix 2. Clinical Laboratory Test	Clarification of laboratory test	

Amendment 2: May 21, 2021
Protocol Version: 1.2
This amendment is considered to be nonsubstantial

Section # and Name	Description of Change	Brief Rationale
Table 3.1: Objectives and corresponding endpoints	Clarification of exploratory objectives	Inclusion of the definition of cellular immunogenicity
All the document	Correction of some typographic mistakes	

Statistical Analysis Plan

Title:

A Phase 2, Comparative, Randomised, Adaptive Trial to Evaluate the safety and immunogenicity of one dose of COMIRNATY® in subjects that had received one dose of VAXZEVRIA® (CombiVacS)

Protocol version

Protocol Code: CombiVacS
Number EudraCT 2021-001978-37
Version: 1.1. Date: April 15, 2021. File: SC_CombiVacS_Protocol_V1.1_20210415.pdf
Version: 1.2. Date: May 21, 2021. File: SC_CombiVacS_Protocol_V1.2_20210521.pdf

Data capture version

Version 3.0. Date April 26, 2021. File: CombiVacS_CRD_v3_20210426.doc

Signature:

USCI Hospital Universitario 12 de Octubre
Name: _David Lora Pablos _ _María Teresa García Morales_ _Agustín Gómez de la Cámara_ Date: _21_ /_MAY_ /_2021_

Coordinator investigator, Hospital Universitario La Paz
Name: _Alberto M Borobia _ Date: _21_ /_MAY_ /_2021_

1 Introduction

1.1 Study rationale and background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causal agent of c (COVID-19) outbreak. Declared as a pandemic on March 11, 2019 by the World Health Organisation, COVID-19 outbreak is an evolving global health emergency. A huge effort for prevention and treatment of COVID-19 has since been devoted globally. As of February 16, 2021, almost 5000 trials related with COVID-19 have been registered.

Vaccination against COVID-19 started on 27 December 2020 across the European Union. Four vaccines have been so far given conditional marketing authorisation by the European Commission: mRNA vaccine BNT162b2 (BioNTech and Pfizer - COMIRNATY), mRNA vaccine CX-024414 (Moderna), adenovirus vaccine ChAdOx1-S (AstraZeneca - VAXZEVRIA) and adenovirus vaccine Ad26.Cov2.S (Janssen-Cilag International NV). These vaccines are indicated for active immunisation to prevent COVID-19 caused by SARS-CoV-2 in individuals 16 or 18 years of age and older. Most coronavirus vaccines are given as two injections: an initial 'prime' dose followed by a 'boost' to stimulate the immune system's memory cells and amplify the immune response (only Ad26.Cov2.S vaccine has been approved as a single-dose). However, their safety and effectiveness using them as heterologous boost regimens have not yet evaluated.

Due this pandemic situation, the ability to mix and match vaccines could make vaccination programmes more flexible, speeding up the process and reducing the impact of any supply-chain disruptions. This concept has been also used in other vaccines, as Ebola one. A heterologous prime-boost combination of Ebola Virus Vaccine was evaluated in healthy adult subjects in a clinical trial, concluding that the standard and accelerated heterologous prime-boost regimens were well tolerated.

and elicited potent cellular and humoral immunogenicity. Therefore, this heterologous prime-boost regimen was approved last year by European regulators to protect against Ebola.

Also, induction of immune response by combining vaccines using different platforms could be stronger. It has been shown that some vaccines produce a better antibodies response while others best stimulate cellular response.

In the case of vaccine against SARS-CoV-2 infection, some trials are ongoing using heterologous prime boost regimen for the prevention of COVID19: 1) NCT04760730 trial use both adenovirus vector vaccines (VAXZEVRIA and rAd26-S), 2) NCT04776317 trial use Chimpanzee Adenovirus serotype 68 (ChAd) and self-amplifying mRNA (SAM) vectors in healthy adult subjects including older adult subjects, and 3) NCT04684446 trial evaluate the safety and immunogenicity of VAXZEVRIA given in combination with (either before or after) rAd26-S.

CombiVacS is the first clinical trial evaluating the immunogenicity and safety of boosted vaccination with a mRNA COVID19 vaccine (COMIRNATY – Pfizer) in subject having received prime vaccination with a viral vector COVID19 vaccine (VAXZEVRIA – AstraZeneca).

1.2 Benefit/ Risk assessment

While the efficacy and safety profile of the approved COVID-19 vaccines are subject to investigation when used as heterologous boost regimen within this protocol, the only contraindication to COVID-19 vaccines has been determined as hypersensitivity to the active substance or to any of the excipients within the vaccine product. Therefore, participants volunteering for this trial are not considered to be at additional risk related to the administration of the COVID-19 vaccine.

Participants in this study are exposed to some general risks. The participants may be subjected to additional blood sampling without representing any harm or discomfort. Collection of other samples, like oropharyngeal or nasal swabs, or urine, are considered not to cause any significant harm.

Participants' identities will be protected, and personal health information (PHI) held securely. No directly identifiable data will be stored in the clinical trial database, and the participant lists will be stored separately, secured, at the local study sites. The risk that unauthorized persons will see the participant's PHI will be kept at a minimum, and measures will be taken to keep PHI confidential and in accordance with the legal requirements.

2 Hypothesis and objectives

2.1 Aim hypothesis

The hypothesis of this trial is that in patients having received a prime dose with VAXZEVRIA immunogenicity after a boosted dose with COMINARTY is better than without booster vaccination.

2.2 Statistical hypothesis

The efficacy hypothesis is:
Ho: G(Y1) / G(Y2) = 1 versus H1: G(Y1) / G(Y2) > 1
With definition:
G(Y1) is the titre of antibodies binding to the SARS-CoV-2 S protein at 14th day after a dose of COMIRNATY in subjects that received a prior single dose of VAXZEVRIA (interventional group).
G(Y2) is the titre of antibodies binding to the SARS-CoV-2 S protein at 14th day in subjects no receiving the COMIRNATY dose at randomization (control group).
Assumption: The serological response to vaccination (antibodies against SARS-Cov2 spike protein) measured by immunoassay, will follow lognormal distribution.

2.3 Objectives and corresponding endpoints

Objectives	Endpoints
Primary Immunogenicity Objective	
To assess the humoral immune response against SARS-Cov-2, 14 days after a vaccination with COMIRNATY in subjects that received a previous single dose of VAXZEVRIA, as compared with no dosing, in SARS-Cov2 seronegative adults.	- Serological response to vaccination (antibodies against SARS-Cov2 spike protein) as measured by immunoassay, 14 days after the boost dose.
Exploratory objective	
To evaluate the relationship between the immune response measured as NAV (Neutralizing antibodies) and antibodies against SARS-Cov2 spike protein measured by immunoassay	- NAV (Neutralizing antibodies) and ELISA (enzyme-linked immunosorbent assay).
Secondary immunogenicity objectives	
To assess the humoral immune response against SARS-Cov-2, 28 days after a vaccination with COMIRNATY in subjects that received a previous single dose of VAXZEVRIA.	- Serological response to vaccination (antibodies against SARS-Cov2 spike protein) as measured by immunoassay, 28 days after the boost dose. - SARS-CoV-2 neutralization as measured by virus neutralization assay, 14 and 28 days after the boost dose.
To assess the long-term (up to 1 year) humoral immune response against SARS-Cov-2 of a dose of COMIRNATY in subjects that received a previous single dose of VAXZEVRIA.	-Serological response to vaccination (antibodies against SARS-Cov2 spike protein) as measured by immunoassay at 3, 6 and 12months after the boost dose. - Neutralizing antibody titers at 3, 6 and 12 months after the boost dose.
To assess the humoral immune response against viral variants of SARS-CoV-2, 14 and 28 days after a dose of COMIRNATY in subjects that received a previous single dose of VAXZEVRIA.	Serological response to vaccination (antibodies against SARS-CoV-2 spike protein) as measured by immunoassay at 3, 6 and 12months after the boost dose.

	- SARS-CoV-2 neutralization as measured by virus neutralization assay at 3, 6 and 12 months after the boost dose.
Secondary efficacy objective	
To assess the occurrence of symptomatic molecularly confirmed COVID-19 and severity of COVID-19 signs and symptoms after the administration of a dose of COMIRNATY in subjects that received a prior single dose of VAXZEVRIA.	-The number of participants with molecularly confirmed COVID-19. - Presence and severity of COVID-19 signs and symptoms as measured by Symptoms of Infection with Coronavirus-19 (SIC).
Safety	
To evaluate the safety of a dose of COMIRNATY in subjects that received a previous single dose of VAXZEVRIA.	- Solicited local and systemic adverse events (AEs) for 7 days after vaccine. - Unsolicited local and systemic adverse events (AEs) for 28 days after vaccine (Two days per week). - Serious adverse events (SAEs) throughout the study (from randomization until end of the study). - Medically-attended adverse events (MAAEs) from the day of vaccination until 6 months after the last vaccination. - Lab parameters: biochemistry and haematology at 1 month visit.

3 Study Design

3.1 Overall Design

This is a randomized, non-blinded, controlled, adaptive, multicenter, Phase II study in subjects aged more than 18 years and in good health or stable clinical situation that have received a previous single dose of VAXZEVRIA.

3.2 Study Interventions and Concomitant Therapy

Study intervention is defined as heterologous boosted strategy based on approved SARS-CoV-2 vaccine intended to be administered to a study participant according to this protocol.

3.2.1 Study Intervention(s) Administered

3.2.1.1 Intervention arm:

The intervention arm (Group 1) will be the arm vaccinated with COMIRNATY after a previous vaccination with VAXZEVRIA between 8 and 12 weeks before the screening visit.

3.2.1.2 Control arm:

The control arm (Group 2) no vaccine will be administered. I If primary analysis at 14 days confirms the starting hypothesis, subjects randomized to no vaccination will be considered for administration of one dose of COMINARTY at day 28th according to Public Health Department of the Ministry of Health recommendations on heterologous vaccination. If vaccinated, subjects will be followed at the time points defined in the flowchart (Group 2).

3.3 Selection sample

A multistage cluster sampling stratified by age groups will be carried out to obtain the number of subjects to be selected from the list of 400,000 subjects registered by Spanish Government as having received only the first dose of AZ vaccine. The first stage will be the autonomous community, the second the postcode and the third the hospital centre. Subjects will be selected in this last stage by systematic sampling stratified by age group (18-49, and 50-59).

3.4 Randomization

The randomization list will be generated using software SAS 9 for Windows. The randomization list will be imported into the REDCap program so the researchers can randomize candidate subjects using an easier-to-use interface. Subjects that meet the selection criteria will be randomized in a 2:1 ratio between the two treatment groups (Intervention arm: administration of a COMINARTY boosted dose vs Control arm: no administration of boosted dose), stratifying by centre, gender and age (18-49 years, and 50-59 years), in order to achieve balanced randomization in the two treatment groups. The assignment of treatment to each subject will be centralized, keeping the sequence hidden.

3.4.1 Blinding

This is an open-label clinical trial.

3.5 Sample Size

Sample size calculation for a log-transformed outcome measure (1) has been performed to assess the humoral immune response against SARS-CoV-2 14 days after dose of COMIRNATY in subjects that received a prior single dose of VAXZERIA, as compared with no dosing. Sample size to obtain will be 600 participants divided in 400 in arm1 and 200 in arm2 (2:1 randomization ratio): we assume that: a) increase in antibodies titre would be at least x1.35 in subjects receiving the dose of COMIRNATY G(Y1) in relation with those not receiving it G(Y2); b) a coefficient of variation equal to 1.2 or 1.0 and similar between arms, c) a 80% statistical power and alpha equal to 0.01 for one side test (H1: G(Y1)/G(Y2) >1). Table for different assumptions in coefficient of variation and G(Y1)/ G(Y2) is displayed in appendix 7.1.

The sample size will be increased by 15% due to possible no-participation.

3.6 Interim analysis

Not aplicable.

3.7 Scientific rationale for study design

There is an urgent need to take decisions about how to proceed in those subjects having received a first dose of VAXZEVRIA during the Covid-19 vaccination campaign in Spain, after the decision taken by public health authorities put on hold the second dose of Vaxzevria. In addition, the ability to mix and match vaccines could make vaccination programmes more flexible, speeding up the process and reducing the impact of any supply-chain disruptions. At this moment no information exists on the safety and immunogenicity response for heterologous vaccination schemes.

Therefore, a pragmatic trial is proposed to obtain enough information for public rapid decision making and to allow long-term evaluation on the safety and immunogenicity response after the boost dose of a mRNA vaccine (COMINARTY) in these subjects.

3.8 Study population

Adult subjects (18 years old or higher) in good health or stable clinical situation volunteering to participate in the trial will be recruited after giving informed consent.
Selection criteria for inclusion of subjects in the trial will be maintained as pragmatic as possible.

3.9 Inclusion and exclusion criteria

3.9.1 Inclusion criteria

Each potential participant must satisfy all of the following criteria to be enrolled in the study:

1. Adult subjects (18 years old) having received a prime VAXZEVRIA vaccination between 8 and 12 weeks before the screening visit.
2. Participants must provide consent indicating that he or she understands the purpose, procedures and potential risks and benefits of the study, and is willing to participate in the study.
3. Subjects in good health or stable clinical situation.
4. Participant is willing and able to adhere to the procedures specified in this protocol.

3.9.2 Exclusion criteria

Any potential participant who meets any of the following criteria will be excluded from participating in the study:

1. Participant has a clinically significant acute illness (this does not include minor illnesses such as diarrhea or mild upper respiratory tract infection) or temperature ≥38.0ºC within 24 hours prior to the planned dose of study vaccine.
2. Participant has a known or suspected allergy or history of anaphylaxis or other serious adverse reactions to COMIRNATY excipients.
3. Subjects with any contraindication to the administration of COMIRNATY, included pregnancy.
4. Subjects with prior documented COVID19 since VAXZEVRIA vaccination.
5. Subjects have symptoms or signs compatible with COVID19.
6. Subjects participating in a clinical trial in the last three months.
7. Any condition or situation precluding or interfering the compliance with the protocol.

3.10 Ethics Committee Approval

The study protocol was evaluated and approved by the Research Ethics Committee for Drugs of the Hospital “La Paz” and Agencia Española del Medicamento (Spanish Agency of Medicienes and Healthcare Products), whose protocol code is 5859.

4 Statistical Analyses

4.1 General considerations

All analyses will be carried out using the statistical software SAS, version 9.4 of the SAS system for Windows.
All continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation (SD), 1st quartile, median, 3rd quartile, maximum and minimum. Absolute and relative frequencies (based on the non-missing sample size) of observed levels will be reported for all categorical variables.
All endpoint continuous variables will be tested for normality hypothesis assumption with the Kolmogorov-Smirnov test. The distribution of the antibodies binding to the SARS-CoV-2 S protein and NAV will be studied using: 1) histogram plot for raw data and for logarithm transformation of raw data, 2) summarize included minimum, 1st percentile, 5st percentile, 25st percentile, 50st percentile, 75st percentile, 95st percentile, 99st percentile and maximum, 3) quantile-quantile and probability plot for raw data (versus lognormal distribution) and for logarithm transformation of raw data (versus normal distribution).
The number of participants and the flowchart of the study will be presented. The period of enrolment and the total number of participants screened to be included in the study will be presented. The number of ineligible participants and the total number of randomized participants will be presented. The number of participants who never receive the study intervention will also be presented by treatment group and those who were included in the full analysis set and the safety analysis set will be presented (Participant flowchart).
All describe analysis will be realized by study arms.

4.2 Analysis sets

For the purposes of analysis, the following analysis sets are defined:

Participant Analysis Set	Description
Full analysis set	All participants having received at least one dose of study intervention and who have at least one efficacy evaluation after baseline. Participants will be included in the analyses according to the planned randomized intervention
Safety analysis set	All participants who are exposed to a study intervention. Participants will be analysed according to the intervention they actually received

Table 1

Additional details:

- The full analysis set is used to analyse endpoints related to the efficacy objectives and the safety analysis set is used to analyse the endpoints and assessments related to safety.
- Full analysis set is used to estimate the primary endpoints and the secondary endpoints for secondary core objective.
- Full analysis set is used to for sensitivity analyses.
- Safety analysis set and full analysis set are used to present safety data.

4.3 Participant flowchart

Flow diagram of the progress through the phases of a parallel randomised trial of two groups (that is, enrolment, intervention allocation, follow-up, and data analysis) will be described.

4.4 Baseline characteristics

Screening visit, lower or equal to 7 days before randomization, for all patient who signed the informed consent will be described.
The baseline characteristics will be considered as the information provided at visit 1. Baseline characteristics will be presented by study arm. Full analysis set.

4.5 Evaluation of study objectives

4.5.1 Efficacy objectives

4.5.1.1 Primary efficacy endpoint

Full analysis set.
Total sample sizes and number of patients with quantifiable serological response to vaccination (antibodies against SARS-Cov2 spike protein) at 14 days after the boost dose will be displayed.
Serological response to vaccination (antibodies against SARS-Cov2 spike protein) at 14 days after the boost dose will be graphed using histogram plot.
Assuming that the range of serological response to vaccination (antibodies against SARS-Cov2 spike protein) at 14 days after the boost dose over which they can be quantified is restricted by technical limitations in upper values, and too, assuming that serological response (raw data) has lognormal distribution. The following analysis will be realized:
Parametric survival model (2,3) will consider the right censoring values and raw data with distribution lognormal data (equal to logarithm transformation data with distribution normal), where antibodies against SARS-CoV2 spike protein will be response variable and treatment effect will be evaluated (interventional group versus control group). Additional post-treatment ANCOVA adjusting for pre-treatment will be realized (4), i.e, baseline immunity values will be added as covariable. Lastly, age, sex and baseline immunity value as covariable will be introduced in the model plus arm group. ANCOVA on change score adjusting for pre-treatment will be realized with the same strategies.
Baseline immunity values no quantified by right value will be replaced by 12500. Upper one-sided 99% Confidence Intervals (CIs) will be calculated using maximum likelihood estimation. P-value lower than 0.01 will show statistical significant.
Check normal distribution assumptions in censored regression models will be realized (3) using a graph of Kaplan-Meier estimate of the cumulative hazard versus the normal (or lognormal) regression model estimate of the cumulative hazard.
Different scenarios will be contemplated (see section 4.7) and sensibility analysis will be realized. A subgroup analysis by sex will also be performed (see section 4.8).

```
*Interventional group will be coded as 1, and control group as 0;
*Values upper 12500 will not be quantified;
*Lower: not missing, upper: missing, lower used as right-censoring value;
*Original variable (raw data) follow lognormal distribution;
proc lifereg data=CombiVacS;
        model (valueLower, valueUpper) = treatment/ alpha = 0.02 distribution=lognormal;
run;
proc lifereg data=CombiVacS;
        model (valueLower, valueUpper) = treatment baseline age sex
        / alpha = 0.02 distribution=lognormal;
run;
*Logarithm transform variable (log raw data) follow normal distribution;
proc lifereg data=CombiVacS;
        model (logvalueLower, logvalueUpper) = treatment/ alpha = 0.02 distribution=normal;
run;
```

4.5.1.1.1 Upper and lower limits

RBD (ROCHE)
Lower limit: 0,4 BAU/mL Upper limit without dilution: 250 BAU/mL Upper limit with dilution 1/10: 2500 BAU /mL Upper limit with dilution 1/50: 12500 BAU /mL **Cut off: 0,80 BAU/mL** **Equal or upper to 0,80 Positive, lower Negative**
Trimerica (DIASORIN)
Lower limit: 4,81 BAU/mL Upper limit without dilution: 2.080BAU/mL Upper limit with dilution 1/20 : 41.600 BAU /mL **Cut off: 33.8 BAU/mL** **Equal or upper to 33.8 Positive, lower Negative**

4.5.1.2 Secondary efficiency objectives

All analysis will done with full analysis set.

Serological response to vaccination (antibodies against SARS-Cov2 spike protein) at 7 and 28 days, 3, 6 and 12 months after the boost dose will be realized equal to previous section (4.5.1.1).

SARS-CoV-2 neutralizing antibodies as measured by virus neutralization assay, i.e, neutralizing antibody (NAV), at 14 and 28 days, 3, 6 and 12 months after the boost dose will be realized equal to previous section (4.5.1.1).

The absolute and relative frequencies of individuals will be used to describe molecularly confirmed COVID-19, presence and severity of COVID-19 signs and symptoms as measured by Symptoms of Infection with Coronavirus-19 (SIC). For each dichotomic variable will be compared between study arms through the two-tailed chi-square test with alpha error equal to 0,05, and risk ratio with 95% Confidence Interval will be calculated.

*Interventional group will be coded equal to 1, and control group equal to 0;
*Assumption: dichotomic variable will be coded equal to 1 for yes: presence;

```
proc sort data=combiVacdata;
        by descending dichotomic descending group;
run;
proc freq data= combiVacdata order = data;
        table dichotomic * group / chisq relrisk;
run;
```

Immunology cellular in baseline and 14 after the boost dose will be realized equal to previous section (4.5.1.1).

4.5.2 Safety objectives

All analysis will be based on the full and safety analysis sets.

The absolute and relative frequencies of individuals will be used to describe solicited local and systemic adverse events (AEs) for 7 days after vaccine by study arms.

The absolute and relative frequencies of individuals will be used to describe unsolicited local and systemic adverse events (AEs) for 28 days after vaccine by study arms.

The absolute and relative frequencies of individuals will be used to describe serious adverse events (SAEs) throughout the study (from randomization until end of the study) by study arms.

The absolute and relative frequencies of individuals will be used to describe medically-attended adverse events (MAAEs) from the day of vaccination until 6 months after the last vaccination by study arms.

4.6 Exploratory analysis

All analysis will be based on the full analysis set.

Correlation of the immune response measured by NAV and ELISA with original measures will be studied through Spearman's correlation coefficient in each time point: 14 and 28 days, 3, 6 and 12 months after the boost doce. Scatterplot will be displayed for each time point. Axis will be transformed to logarithm measures.
Change in immune response from baseline (randomization visit) over the year (7 days, 14 days, 28 days, 3 months, 6 months and 12 months) after COMIRNATY vaccination in the immediate and control groups will be studied using a longitudinal model where the influence of each individual on their repeated outcomes is allowed. Mixed-effects regression models for continuous outcomes will be used (4). The variable dependent will be the logarithm transformation of serological response to vaccination (antibodies against SARS-Cov2 spike protein), and will be adjusted by visit and study arms. Random intercept and trend will be explored, and compound symmetry structure of covariance pattern models will be used. Change in the time will graphed.
Also, assuming that the range of serological response to vaccination at specific time after the boost dose over which they can be quantified is restricted by technical limitations in upper values, change in immune response will be evaluated using mixed models for longitudinal right-censored repeated measures (5) with procedure NLMIXED of SAS.
The absolute and relative frequencies of individuals will be used to describe the patients reaching a FRNT50 GMTs >1000 at 14 and 28 days, at 3, 6 and 12 months. The dichotomous variable will be compared between study arms using the two-tailed chi-square test with alpha error equal to 0.05, and risk ratio with 95% Confidence Interval will be calculated.

4.7 Sensitivity analysis

Full analysis set.
This analysis will be realized for serological response to vaccination (antibodies against SARS-Cov2 spike protein) and for SARS-CoV-2 neutralising antibodies measured as NAV at specific time after the boost dose.

4.7.1 Uncensored analysis

All value that can not be quantified by technical limitation will be eliminated.
Geometric means will be calculated for the raw data with lognormal distribution, skewed and positive values (Tabla 53), and mean for logarithm transformation (Tabla 54). For raw data with lognormal distribution, the Coefficient of Variation (CV) will be the measure of variability, and the standard deviation will be the measures for logarithm transformation. The assumption of equal CV will be tested analogously to the assumption of equal variances on the normal scale. When the CV of two study arms are assumed equal (p-value upper 0.05), the pooled estimate of variability will be used and satterthwaite estimate, in other case (p-value <0.05). Upper one-sided 99% Confidence Intervals (CIs) will be calculated using t-distribution. P-value lower than 0.01 will show statistical significant, i.e, ratio measures of interventional group divided control group is greater than 1.

```
*Interventional group will be coded as 1, and control group as 0;
* original variable follow lognormal distribution;
proc sort data= combiVacdata;
        by descending group;
run;
proc ttest data= combiVacdata test=ratio sides=U alpha=0.01 order=data;
        class group;
        var originalvariable;
run;
```

Additional, analysis of the serological response to vaccination (antibodies against the SARS-Cov2 peak protein) between the control and intervention groups will be adjusted for age, sex and baseline immunity values using a longitudinal regression model (4): analysis of covariance of post-test score.
By other side, raw data will be described using median and 25% and 75% percentile, and Mann-Whitney-Wilcoxon test will be used (Tabla 55).

```
*Mann-Whitney-Wilcoxon test;
*No option about one or two sided. P-value lower than 0.02 will be considered statistical significant;
proc npar1way data= combiVacdata wilcoxon;
        class group;
        var originalvariable;
run;
```

4.7.2 Censored analysis

Assuming that the range of serological response to vaccination at specific time after the boost dose over which they can be quantified is restricted by technical limitations in upper values, Kaplan-Meier analyses will be used to estimate the cumulative frequency distribution of the magnitude of serological response increment by intervention and control group,

being the estimated proportion of patients with serological response increase upper than any given value. Based on the Kaplan-Meier estimate, the median reduction in serological response level can be estimated, corresponding to the magnitude of increase that 50% of patients would be expected to exceed (2,3). Percentile 25, 50 and 75 will be estimated with interval confidence.

4.7.3 Censored analysis without parametric assumptions

Other methods would be evaluated when do not make any parametric assumptions about the distribution of serological response , so-called accelerated failure time models for time-to-event data (3,6).

4.7.4 Dichotomizing continuous response

Additional, the change of serological response could be dichotomized under different cut-off: 1) upper 30% of change versus lower 30% of change; 2) upper 50% of change versus lower 50% of change. The absolute and relative frequencies of individuals will be used to describe patient with upper and lower cut-off values by arm study. For each dichotomic variable (cut-off) will be compared between study arms through the two-tailed chi-square test with alpha error equal to 0,05, and risk ratio with 95% Confidence Interval will be calculated. Dependent variable will be adjusted by age, sex and basal serological response.

4.8 Subgroup analysis

A subgroup analysis by sex, and age groups will be performed for the primary efficacy endpoint: Serological response to vaccination (antibodies against SARS-Cov2 spike protein) and for the secondary efficacy endpoint: Serological response to vaccination (SARS-CoV-2 neutralising antibodies (NAV)).
The data sets specified in the sections for each objective will be used for these analyses.

4.9 Intermediate statistical analysis

Not aplicable.

4.10 Missing data imputation

Missing value will not be imputed.
Trimerica (DIASORIN) with value lower than 4.81 BAU/mL will be replaced by 2.405, i.e, lower limit divided by 2.
RBD (ROCHE) with value lower than 0.4 BAU/mL will be replaced by 0.2, i.e, lower limit divided by 2.

5 Glossary

AEs: adverse events
ANCOVA: Analysis of covariance
CIs: Confidence Intervals
COVID-19: coronavirus disease 2019
Diff: Difference Mean
ELISA: enzyme-linked immunosorbent assay
GM: Geometric Mean
MAAEs: Medically-attended adverse events
NAV: Neutralizing antibody
PHI: personal health information
RR: Risk Ratio
SAEs: Serious adverse events
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2
SIC: Symptoms of Infection with Coronavirus-19
Std Dev: Standard Deviation

6 References

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7 Apendix

7.1 Sample size

7.1.1 Programing code

The code was executed in SAS versión 9.4.

```
%LET mialfa = 0.01;
%LET mibeta = 0.20;
%LET mir = 2;
%LET mimissing = 0.15;
data miwolfe;
 *WOLFE R. CONTROL CLIN TRIALS 1999. 20:547-554;
 alfa = &mialfa;
 beta = &mibeta;
 r = &mir;
 do k2 = 1.2 to 1.0 by -0.1;
  k1 = k2;
  do gammarat = 1.35 to 1.5 by 0.05;
   numerador = (log(1 + k2**2) + (log(1 + k1**2)/r)) * ( quantile("normal",
alfa/2) + quantile("normal", beta)  )**2;
   denominador = (log(gammarat) - (log(1 + k2**2) - log(1 + k1**2))/2)**2;
   n2 = numerador / denominador;
   n1 = r*n2;
   mn2 = ceil(n2 / (1 - &mimissing));
   mn1 = ceil(n1 / (1 - &mimissing));
   total = mn2 + mn1;
   output;
  end;
 end;
run;
proc print data=miwolfe label;
  var alfa beta r k2 gammarat n2 n1 mn2 mn1 total;
  label r="2:1" k2="Coef. Var" gammarat="G(Y1)/G(Y2)"
  n1="COMIRNATY group (n=)" n2="VAXZERIA group (n=)"
  mn1="COMIRNATY group (n=) with 15%"
  mn2="VAXZERIA group (n=) with 15%" total="N";
  FOOTNOTE "Report for &sysday, &sysdate. SAS version: &sysver";
run;
```

7.1.2 Output

Report of sample size by SAS 9.4

Obs	alfa	beta	2:1	Coef. Var	G(Y1)/G(Y2)	VAXZERIA arm (n=)	COMIRNATY arm (n=)	VAXZERIA arm (n=) with 15%	COMIRNATY arm (n=) with 15%	N
1	0.01	0.2	2	1.2	1.35	173.506	347.012	205	409	614
2	0.01	0.2	2	1.2	1.40	138.026	276.052	163	325	488
3	0.01	0.2	2	1.2	1.45	113.186	226.372	134	267	401
4	0.01	0.2	2	1.2	1.50	95.050	190.100	112	224	336
5	0.01	0.2	2	1.1	1.35	154.248	308.496	182	363	545
6	0.01	0.2	2	1.1	1.40	122.706	245.412	145	289	434
7	0.01	0.2	2	1.1	1.45	100.623	201.247	119	237	356
8	0.01	0.2	2	1.1	1.50	84.500	169.000	100	199	299

Obs	alfa	beta	2:1	Coef. Var	G(Y1)/G(Y2)	VAXZERIA arm (n=)	COMIRNATY arm (n=)	VAXZERIA arm (n=) with 15%	COMIRNATY arm (n=) with 15%	N
9	0.01	0.2	2	1.0	1.35	134.827	269.653	159	318	477
10	0.01	0.2	2	1.0	1.40	107.256	214.513	127	253	380
11	0.01	0.2	2	1.0	1.45	87.954	175.908	104	207	311
12	0.01	0.2	2	1.0	1.50	73.861	147.722	87	174	261

7.1.3 Other code

Sample size for default implement in PROC POWER of SAS version 9.4.

```
proc power;
 twosamplemeans test = ratio
  meanratio = 1.35 to 1.5 by 0.05
  alpha = 0.01
  power = 0.80
  cv = 1.2 to 1 by -0.1
  groupweights = 2 | 1
  ntotal = .;
  plot x=effect step=0.05;
run;
```

7.1.4 After clinical trial evaluation

Non-significant result in the primary efficacy endpoint will be evaluated using pretrial perspectives, sample size assumptions, versus post-trial perspectives, observed difference with confidence intervals (7,8).