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A phase I, needle free, dose escalation clinical trial of pEVAC-PS, a candidate pan-Sarbecovirus Vaccine

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SUMMARY

Background: Coronaviruses such as SARS, SARS-CoV-2 and related Sarbeco-Coronaviruses continue to pose global health threats, underscoring the need for vaccines capable of inducing broad cross-sarbecovirus protection. The pEVAC-PS vaccine was developed using Digitally Immune Optimised Synthetic Vaccine (DIOSynVax) technology and pre-clinically selected for the ability to induce broadly protective immune responses across the Sarbecoviruses including SARS, SARS-CoV-2, and related viruses representing potential zoonotic spillovers. For this first-in-human study, the antigen was delivered as a DNA vaccine to enable thermostability and needle-free intradermal administration to support future deployment in resource-limited settings.

Methods: This open label phase I dose escalation study investigated the safety, tolerability and immunogenicity of the pEVAC-PS vaccine candidate against SARS, SARS-CoV-2 and related Sarbeco Coronaviruses via needle-free intra-dermal delivery using the PharmaJet Tropis Device. Healthy volunteers aged 18 to 50 who had received two or three prior doses of COVID-19 vaccine, and without recent confirmed COVID-19 infection, were enrolled sequentially to receive a dose escalation regime of 0.2 mg, 0.4 mg, 0.8 mg, and 1.2 mg of pEVAC-PS, administered at day zero and day 28. The primary outcomes were safety and reactogenicity, documented by solicited and unsolicited adverse events, serious adverse events and adverse events of special interest. Secondary outcomes were immunogenicity measured primarily by humoral responses to SARS-CoV-1 and SARS-CoV-2 antigens at day 56 (28 days after the second dose of vaccine). International Clinical Trials Registry Platform registered, ISRCTN87813400.

Findings: Between December 2021 and September 2023, a total of 39 volunteers were vaccinated. The vaccine was well tolerated at all four doses with no significant safety concerns elicited. Interpretation of immunogenicity outcomes was influenced by high baseline antibody levels and heterogeneous exposure histories due to ongoing waves of Omicron variant infections during recruitment, which differed across dose-escalation cohorts and introduced unavoidable immune bias.

Interpretation: Needle-free intradermal delivery of this novel computationally designed PanSarbeco vaccine was safe and well tolerated. Although immunogenicity was modest in the context of substantial pre-

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existing immunity, participants developed measurable responses to conserved, vaccine-encoded sarbecovirus epitopes, supporting the feasibility of this antigen design strategy.
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Introduction

In the past 20 years, there have been three outbreaks caused by betacoronaviruses, two from the *sarbecovirus* subgenus and one from *Merbecovirus*.[1] A large reservoir of sarbecoviruses in bats have potential to spill-over through binding to the ACE2 human receptor, with the potential of causing further pandemics.[2] In 2002/2004 the sarbecovirus SARS-CoV-1 led to the SARS outbreak, which originated in China and resulted to over 700 deaths across nearly 30 countries.[3] In 2012, MERS-CoV (Merbecovirus) was first detected in Jordan, and subsequent outbreaks, predominantly in the Arabian Peninsula, have caused around 2200 cases and 790 deaths, largely through zoonotic transmission from camels.[4,5] Most recently, SARS-CoV-2 (Sarbecovirus) has caused the ongoing COVID-19 pandemic, resulting in more than 7 million deaths globally between 2020 and 2023, impacting every country around the world.[6] The continued circulation of SARS-CoV-2 has resulted in the continuous emergence of new variants of the virus with mutations leading to varying degrees of immune escape, causing recurrent waves of infection despite a substantial degree of immunity from infection and vaccination.[7]

Although COVID-19 vaccines are now being updated periodically to better align with the currently circulating variants, the time required for manufacturing and distributing these updated vaccines has led to a situation where the vaccines in use often do not correspond to the dominant variant or subvariant in circulation.[8] Booster vaccination has so far proven effective at bolstering protection against severe illness even in the face of a mismatch of the precise antigen included. However, there remains a need for vaccines which can both provide broader coverage against current and potentially more dangerous future mutations of SARS-CoV-2 and against the future emergence of different sarbecoviruses that may pose a pandemic threat.[9]

Novel technology to design broad coverage, pan-sarbecovirus vaccines has been created utilising antigen libraries for screening to identify conserved sub-structures within the receptor binding domain of the spike protein found in sarbecoviruses. This approach aims to elicit robust and targeted immune responses. High throughput screening combined within vivo pre-clinical selection based on immunogenicity, has led to the identification of the vaccine pEVAC-PS. The vaccine encodes a computationally designed, receptor-binding domain (RBD) sequence incorporating conserved structural motifs across sarbecoviruses that confer the ability to neutralise SARS-CoV-1, SARS-CoV-2 and other bat sarbecoviruses.[10,11] The vaccine was formulated as DNA to facilitate optimal delivered via needle free technology. This feature is particularly relevant for low- and middle-income settings where cold chain and the transport and disposal of medical sharps can be limiting factors in vaccine distribution.

This study represents the first human dose escalation trial of the candidate pan-Sarbecovirus vaccine, pEVAC-PS, aimed at assessing its safety, reactogenicity, and immunogenicity across four different dose levels.

Methods

Study design and participants

This phase I, first in human dose escalation trial was conducted in healthy adults aged 18 to 50 years who had received two or three doses of COVID-19 vaccine, with the most recent dose having been no sooner than 84 days previously, and without confirmed COVID-19 infection within the previous 180 days. A full list of inclusion and exclusion criteria can be found in the study protocol (supplementary appendix page 9). Participants were recruited at the NIHR Southampton Clinical Research Facility at the University Hospital Southampton NHS Foundation Trust, and from April 2023 at NIHR Cambridge Clinical Research Facility at Addenbrookes Hospital, Cambridge.

All participants provided written informed consent. Eligible participants were recruited in a stepwise, dose escalation manner to one of four groups, receiving two doses of pEVAC-PS at 0.2 mg, 0.4 mg, 0.8 mg or 1.2 mg. Participants received an initial vaccination at Day 0 (Week 1), and a second dose at Day 28 (Week 4), with follow-up immunogenicity assessments conducted at Days 0, 28 (pre-dose 2), 42 (Week 6), and 56 (Week 8).

Prior to dose escalation, all participants in the current group had to have received their first dose of vaccine, with 72 h having passed since the final participant received their first dose. At this point a safety review was performed and the decision to dose escalated was made by the independent local safety monitoring committee.

Due to the stepwise dose escalation design, and the need for differing numbers of administrations at each dose level, it was not feasible to blind participants or investigators to group allocation. The safety and immunogenicity outcomes for phase I were considered robust to an open label design. Safety analysis was not blinded due to the charter for safety committee and investigator joint review of data at each step specified. The safety committee decision regarding continuation was made without the investigators present, after detailed discussion of the safety data on a per participant basis during this dose escalation trial.

The study protocol and documents were reviewed and approved by the South Central-Berkshire Research Ethics Committee, University Hospital Southampton, and the Medicines and Healthcare Products Regulatory Agency (Eudra CT 2021–002227-38, REC reference 21/SC/0337). The trial was registered with ISRCTN, number 87813400.

Procedures

pEVAC-PS was allowed to thaw to room temperature and administered as soon as possible (within 2 h) intradermally using the PharmaJet Tropis. 0.2 mg doses were given as a single administration into one deltoid muscle, whereas doses of 0.4 mg, 0.8 mg and 1.2 mg were given as two, four and six administrations respectively, divided equally between both deltoid muscles.

The injection site was covered with a sterile dressing and volunteers were observed for 60 min following vaccine administration. A telephone follow up visit was arranged for 24 h post vaccination, and in person visits were scheduled for days 3, 7, 14, 28, 42, 56, 84 and 182, with an optional visit at day 365. The second dose of vaccine was administered at the day 28 follow up visit.

Participants were asked to document their temperature and any solicited local and systemic adverse events (AEs) for the 7 days following each vaccination using a paper diary, and unsolicited adverse events for 28 days following each vaccination. Reviews of all AEs were conducted at each follow up visits. Serious Adverse Events (SAEs) and Adverse Events of Special Interest (AESIs) were recorded for the duration of follow up. Blood tests for safety assessment were

performed at day 0, 3, 7, 28 and 42, including full blood count, renal function and liver function tests. Grading of AEs for severity and assessment of causality were conducted according to guidelines included in the study protocol. Unsolicited AEs were coded according to MedDRA (MedDRA® the Medical Dictionary for Regulatory Activities terminology).

Blood samples for serological and exploratory immunology assessments were collected alongside those for safety assessments. Humoral responses to the vaccine were assessed using several methods, including standardises total IgG ELISA towards the RBD of SARS-CoV-1, SARS-CoV-2 Wuhan strain and the vaccine construct (produced in-house), pseudotype-based microneutralisation assays, peptide microarray analysis and automated western immunoblotting to measure antibody binding to SARS-CoV-2 nucleocapsid antigen (as a screening tool for sub-clinical SARS-CoV-2 infection).

Laboratory staff were not blinded, but samples were analysed by batch analysis without bias for groups or individual volunteers.

Outcomes

The main objective of this study was to evaluate the safety and reactogenicity of pEVAC-PS when administered as a booster in healthy volunteers. This was measured by the incidence of solicited systemic and local reactogenicity signs and symptoms for seven days post-vaccination, as well as unsolicited adverse events (AEs) for 28 days following vaccination. Additionally, changes from baseline in safety laboratory measures and the occurrence of disease enhancement episodes were assessed. The secondary objective was to assess the humoral immunogenicity of pEVAC-PS through the analysis of SARS-CoV and SARS-CoV-2 RBD and vaccine construct RBD (namely PanSarbeco RBD) antibody titres, with the primary immunogenicity endpoint occurring at day 56 (Week 8).

Statistical analysis

Safety data were analysed using R (version 4.3.0, R Foundation) and R Studio software (2024.04.2+74). Means and standard deviations are used for demographic groups and for plotting trends in safety blood tests. The safety analysis population consisted of all participants who received at least one dose of the pEVAC-PS vaccine.

The laboratory analysis population consisted of participants who received two doses of pEVAC-PS and did not test positive for COVID-19 prior to day 56 (Week 8). Measurements of immunological endpoints are presented as median with interquartile range (IQR) of log_{10}-transformed EC_{50} values for ELISA and log_{10}-transformed IC50 values for neutralisation. The Friedman test was used to assess changes in ELISA or neutralizing responses over time, across antigens (for ELISA) or pseudoviruses (for neutralisation), with Dunn's post-hoc multiple comparisons test applied to identify significant pairwise differences. A Mann–Whitney test was used to compare $log_{10}EC_{50}$ values at Day 56 (Week 8) and Day 84 (Week 12) in Supplementary Figure 3. All statistical analyses were performed using GraphPad Prism 10.4.0.

Role of the funding source

This research was funded by Innovate UK (Project Reference: 72845), covering the period from August 2020 to March 2023.

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The authors had full access to all the data in the study and were responsible for the decision to submit for publication.

Results

The study was performed between December 2021 and September 2023, and 39 of the 180 volunteers who were screened for eligibility were enrolled in the study (Fig. 1: ***CONSORT diagram***). Four participants in group one tested positive for COVID-19 between the first and second vaccine, and so were removed from the primary immunogenicity analysis and did not receive a second dose of vaccine. An additional participant was found to have been enrolled in error into group one due to a mistake in laboratory processing of their screening bloods and was withdrawn following their first dose. A further participant in group 1 tested positive for COVID-19 between their second dose of vaccine and the day 56 follow up visit and was removed from the primary immunogenicity analysis. As the protocol stipulated a minimum of 6 participants per group were needed for the immunogenicity analysis, a further 3 participants were enrolled in group 1 and received 2 doses of vaccine. As a result, 12 participants in group 1 received the first dose of vaccine, and 7 participants received two doses of vaccine. All immunised participants were included in the safety analysis. Only the 6 participants who received both doses and did not test positive for COVID-19 by the day 56 follow up visit were included in the immunogenicity analysis. In group 3, one participant tested positive for COVID-19 between the first and second dose and so was removed from the primary immunogenicity analysis and did not receive a second dose of vaccine. In groups 2 and 4 all 9 enrolled participants received both doses of vaccine and were included in the safety and immunogenicity analysis.

Baseline demographic data of the 39 participants included in the safety analysis are summarised in Table 1***: Baseline demographic data***.

Group 1 participants were recruited and immunised during the Omicron BA.1 peak between December 2021 and March 2022. Participants in Group 2 were recruited during Omicron BA.2 and BA.5 peaks throughout a period of 7 months from May to November 2022. Two participants in Group 3 were recruited at the end of 2022 under the dome of BA.5 and the remaining participants in Group 3 and Group 4 were all recruited when XBB sub-variants were still prevalent between May and September 2023 at the time of which three participants were recruited in Group 1 as replacement to infected participants as mentioned earlier. The antibody responses measured over eight weeks reflected a combination of vaccine-induced immunity and potential pre-exposure to circulating variants (Fig. 2***: Vaccination and SARS-CoV-2 variant timeline***).

No serious adverse reactions (SARs), suspected unexpected adverse reactions (SUSARs) or serious adverse events (SAEs) occurred. There were 15 adverse events of special interest, all of which were COVID-19 episodes which were of grade one or two severity and did not require medical attention. There were 121 unsolicited adverse events, all of which were grade one or two severity and 23 were deemed possibly, probably or definitely related to the vaccine. There were 12 laboratory adverse events considered clinically significant, all of which were grade one or two severity and self-resolved without intervention during the study. No clear changes in haematological or biochemical markers were detected over time (supp Fig. 1).

All four dose concentrations of pEVAC-PS were generally well tolerated. No grade three local solicited adverse events were experienced. There were seven grade three systemic solicited adverse events experienced in group one, all of which occurred in one participant and were considered most likely due to intercurrent COVID-19 illness experienced coincidentally within the week following vaccination (Fig. 3**: Reactogenicity**). There was no clear dose effect in increasing reactogenicity. There were fewer solicited adverse events following second doses than first doses (e.g., 14 grade two

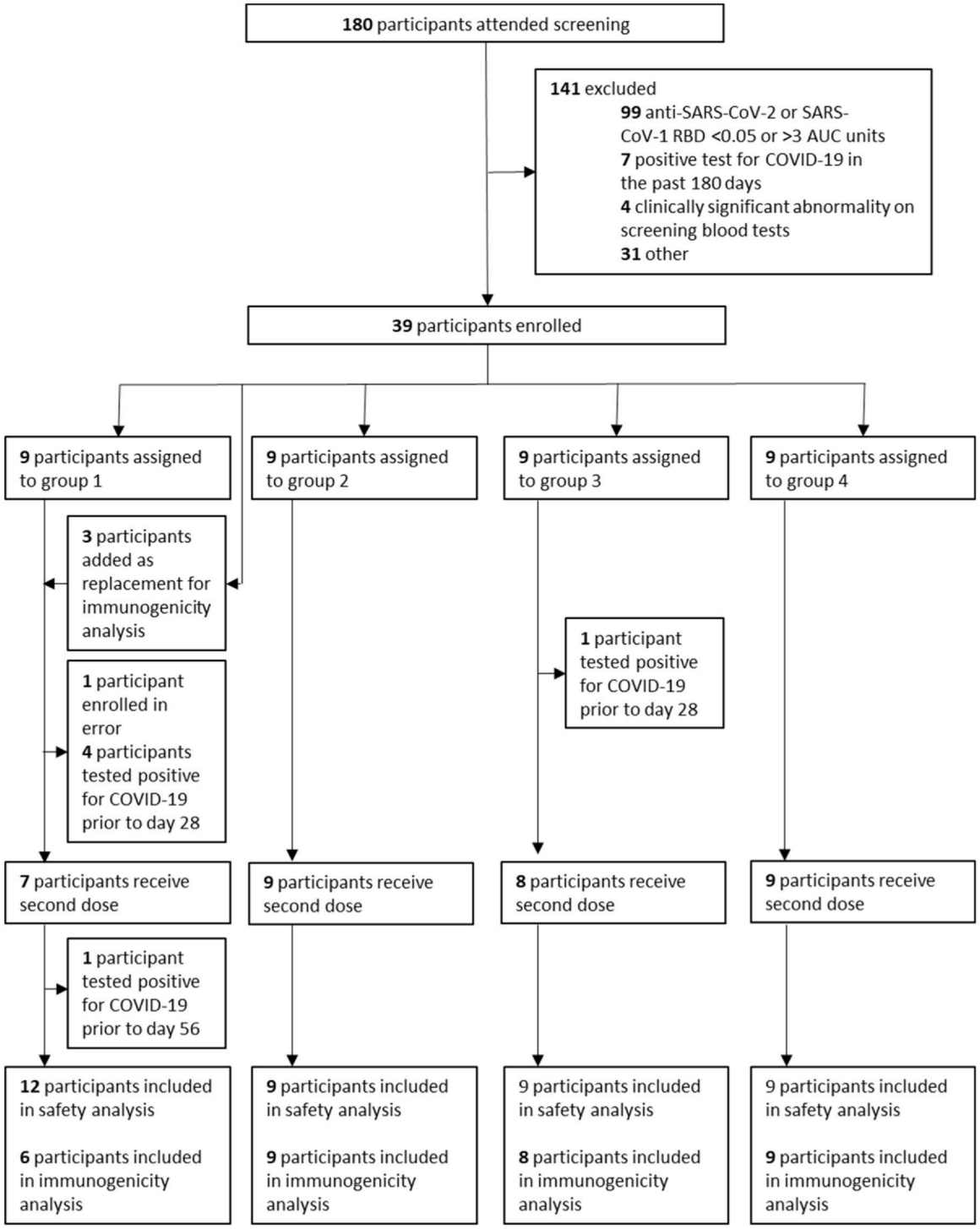

Fig. 1. CONSORT diagram.

adverse events following dose one compared to three grade two adverse events following dose two).

Prior to the evaluation of the immunogenicity of the vaccine, serum samples collected from participants at days 0 (Week 1), 14 (Week 2), 28 (Week 4), 42(Week 6), and 56 (Week 8), were screened for evidence of prior SARS-CoV-2 infection. This was performed using the Jess Automated Western Blotting system, which detects increases in nucleocapsid antibodies indicative of virus exposure during the vaccination trial. It should be noted that reliance on anti-N IgG detection for identifying asymptomatic infection episodes is limited and may have missed some infections that did not elicit detectable N-specific IgG responses.

Based on this analysis, serum samples from two participants in Group 2 (supp Fig. 2) and one in Group 4 were excluded from subsequent primary immunogenicity analysis as demonstrated by an increase in SARS-CoV-2 nucleocapsid protein antibodies.

Following the exclusion of participants with evidence of intercurrent SARS-CoV-2 infection, binding antibody responses were assessed as the primary immunogenicity endpoint. Serum samples were analyzed for reactivity to the PanSarbeco Vaccine RBD, SARS-

Table 1
Baseline demographic data.

		Group 1 0.2 mg (N = 12)	Group 2 0.4 mg (N = 9)	Group 3 0.8 mg (N = 9)	Group 4 1.2 mg (N = 9)	Overall (N = 39)
Age (years)	Mean, SD	33.2(11.8)	42.8(9.0)	37.8(10.1)	37.4(10.6)	37.4(10.7)
Weight (kg)	Mean, SD	84.6(11.1)	80.5(13.6)	77(15.5)	72.7(9.1)	79.1(12.8)
Sex (N, %)	Male	6(50)	4(44.4)	4(44.4)	4(44.4)	18(46.2)
	Female	6(50)	5(55.6)	5(55.6)	5(55.6)	21(53.8)
Ethnicity (N, %)	Arab	0(0)	0(0)	0(0)	0(0)	0(0)
	Asian or Asian British	1(8.3)	1(11.1)	0(0)	2(22.2)	4(10.3)
	Black or Black British	2(16.7)	0(0)	0(0)	0(0)	2(5.2)
	Mixed	0(0)	0(0)	0(0)	1(11.1)	1(2.6)
	White	9(75)	8(88.9)	9(100)	6(66.7)	32(82.1)
	Other	0(0)	0(0)	0(0)	0(0)	0(0)
Smoking (N, %)	Current	0(0)	0(0)	0(0)	1(11.1)	1(2.6)
	Past	1(8.3)	2(33.3)	3(33.3)	4(44.4)	11(28.2)
	Never	11(91.7)	6(66.7)	6(66.7)	4(44.4)	27(69.2)
Concomitant Medication (N, %)	Yes	9(75)	6(66.7)	8(88.9)	5(55.6)	28(71.8)
	No	3(25)	3(33.3)	1(11.1)	4(44.4)	11(28.2)
First COVID-19 vaccine (N, %)	ChAdOx1-nC0V19	5(41.7)	5(55.6)	5(55.6)	4(44.4)	19(48.7)
	BNT162b2	7(58.3)	4(44.4)	4(44.4)	4(44.4)	19(48.7)
	mRNA1273	0(0)	0(0)	0(0)	1(11.1)	1(2.6)
Second COVID-19 vaccine (N, %)	ChAdOx1-nC0V19	5(41.7)	5(55.6)	5(55.6)	4(44.4)	19(48.7)
	BNT162b2	7(58.3)	4(44.4)	4(44.4)	4(44.4)	19(48.7)
	mRNA1273	0(0)	0(0)	0(0)	1(11.1)	1(2.56)
Third COVID-19 vaccine (N, %)	ChAdOx1-nC0V19	0(0)	0(0)	0(0)	0(0)	0(0)
	BNT162b2	1(8.3)	6(66.7)	6(66.7)	7(77.8)	20(51.3)
	mRNA1273	1(8.3)	1(11.1)	2(22.2)	2(22.2)	6(15.4)
	NA	10(83.3)	2(22.2)	1(11.1)	0(0)	13(33.3)

CoV-2 (Wuhan) RBD, and SARS-CoV-1 RBD using an in-house ELISA. Antibody titres were expressed as $\log_{10}$-transformed EC_{50} values derived from four-parameter logistic curve fits, and their distribution over time is shown in Fig. 4***: Primary immunogenicity assessment***.

Overall, binding titres following vaccination were modest across dose groups. In Group 3 (0.8 mg), a statistically significant increase in SARS-CoV-1 RBD binding titers was observed between week 2 and week 6, and between week 4 and week 6 ($p < 0.05$, Friedman test with Dunn's correction), however, these changes remained within the range of pre-existing responses. In the higher-dose Group 4 (1.2 mg), the only significant change detected was for the vaccine construct RBD between week 1 and week 6 ($p < 0.05$).

Although pEVAC-PS was designed to elicit cross-reactive responses against both SARS-CoV-2 and SARS-CoV-1, this intended boosting effect was not observed. Antibody titers to the SARS-CoV-1 RBD (red violin plots) remained consistently lower than those to the SARS-CoV-2 Wuhan and Vaccine RBDs across all dose groups. Variability between participants was notable, likely reflecting the small group sizes and differences in prior SARS-CoV-2 exposure. In

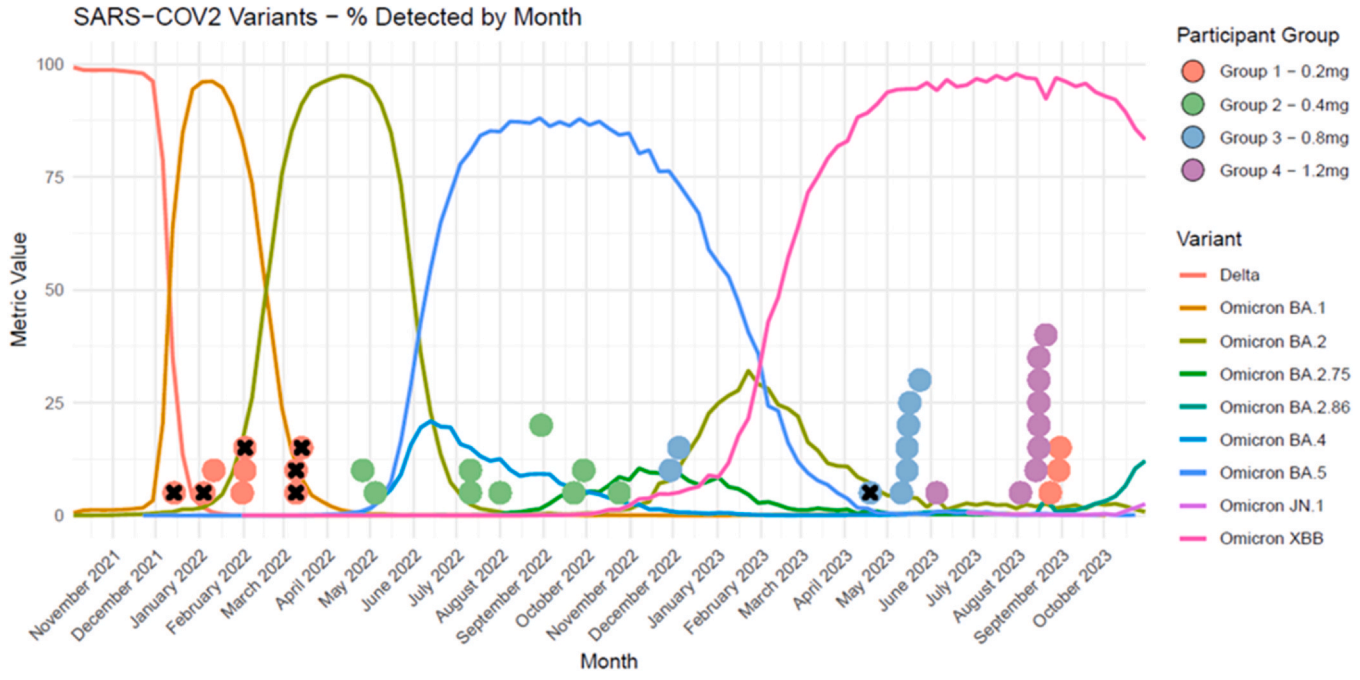

Fig. 2. Vaccination and SARS-CoV-2 variant timeline. The timeline plot shows the vaccinations periods for each group in relation to the dominant SARS-CoV-2 variant circulating in the UK at the time. Dots with an "×" indicate participants who were infected with COVID-19 prior to reaching Day 56 (Week 8), including one participant who was enrolled in error. Epidemiological data on SARS-CoV-2 variant spread was sourced from (https://ukhsa-dashboard.data.gov.uk/respiratory-viruses/covid-19).

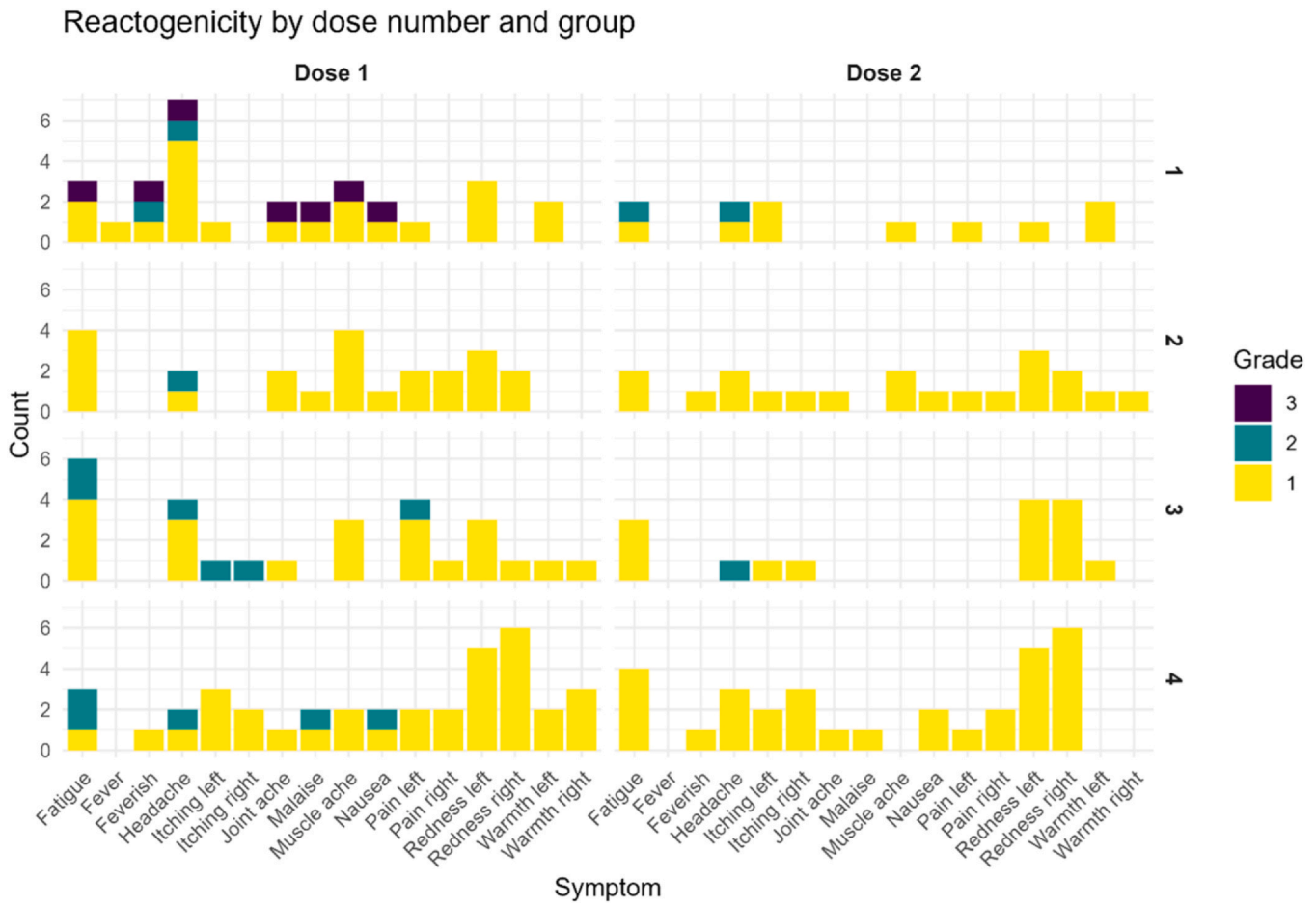

Fig. 3. Reactogenicity. All grade 3 AEs for group 1 were attributed to an intercurrent COVID-19 episode and were not considered related to vaccination.

particular, most participants in the first group (10 of 12) had received two previous SARS-CoV-2 vaccine doses and were enrolled earlier in the pandemic, potentially influencing both baseline and post-vaccination antibody levels.

To assess longer-term antibody persistence, serum samples collected at week 12 (day 84) were compared with those from week 8 (day 56),and tested by ELISA for both SARS-CoV-1 and SARS-CoV-2 RBDs (supp Fig. 3). Statistical comparison using the Mann–Whitney test confirmed no significant differences between the two time points for either antigen or across all groups.

Collectively, these data do not support a robust vaccine-induced increase in antibody responses beyond pre-existing levels. However, increases in binding titers were localised only to the Group 4 (1.2 mg), for the specific vaccine construct, reaching statistical significance by Week 6. Antibody levels remained stable up to week 12, suggesting limited waning within the sampling period.

Neutralizing antibody titers were evaluated in Groups 3 and 4 against pseudoviruses representing SARS-CoV-1, SARS-CoV-2 (Wuhan strain), and SARS-CoV-2 variants Delta and Omicron BA.1 (Fig. 5: Pseudovirus microneutralisation assay). Baseline variability was observed across participants and viral antigens. Neutralizing activity was generally lower against SARS-CoV-2 Wuhan pseudovirus compared to SARS-CoV, and SARS-CoV-2 Delta and Omicron BA.1 variants.

In Group 3, a significant increase in neutralizing activity against Omicron BA.1 was detected between week 1 and week 6 ($p < 0.01$) and between week 1 and week 8 ($p < 0.05$), while in Group 4 a significant increase was observed for the Delta variant between week 1 and week 6 ($p < 0.05$). No other significant changes were detected across time points.

Taken together, pEVAC-PS was safe and well tolerated. Binding antibody responses showed no clear dose-dependent trend and largely remained at baseline levels, with only a minor increases detectable by week 6 in Group 4 (1.2 mg), for the vaccine construct. Neutralizing activity, assessed only in Groups 3 and 4, showed modest increases for specific SARS-CoV-2 variants (Omicron BA.1 in Group 3 and Delta in Group 4), while cross-neutralization to SARS-CoV-1 remained unchanged. Overall, these data indicate limited boosting of pre-existing antibody responses and modest variant-specific neutralization within the vaccination window.

To further characterise the immune responses elicited by the pEVAC-PS vaccine, peptide microarray analysis was performed using sera from six participants (three from Group 2 and three from Group 4). The array comprised overlapping 15-mer peptides spanning the SARS-CoV, SARS-CoV-2, and vaccine construct RBDs, separated by GSG linkers (Supplementary Figure 3A–B).

Comparative analysis of sera collected at Week 1 (Day 0) and Week 8 (Day 56) revealed differential peptide binding profiles following vaccination (Supplementary Figure 3C). Increased reactivity was observed in the N-terminal and ACE2-binding regions of the RBD (Supplementary Figure 3D–F), suggesting enhanced targeting of functionally relevant epitopes.

Notably, antibodies also recognized a conserved region corresponding to the epitope bound by the broadly neutralizing monoclonal antibody S309, consistent with the vaccine's design to elicit cross-sarbecovirus reactivity.

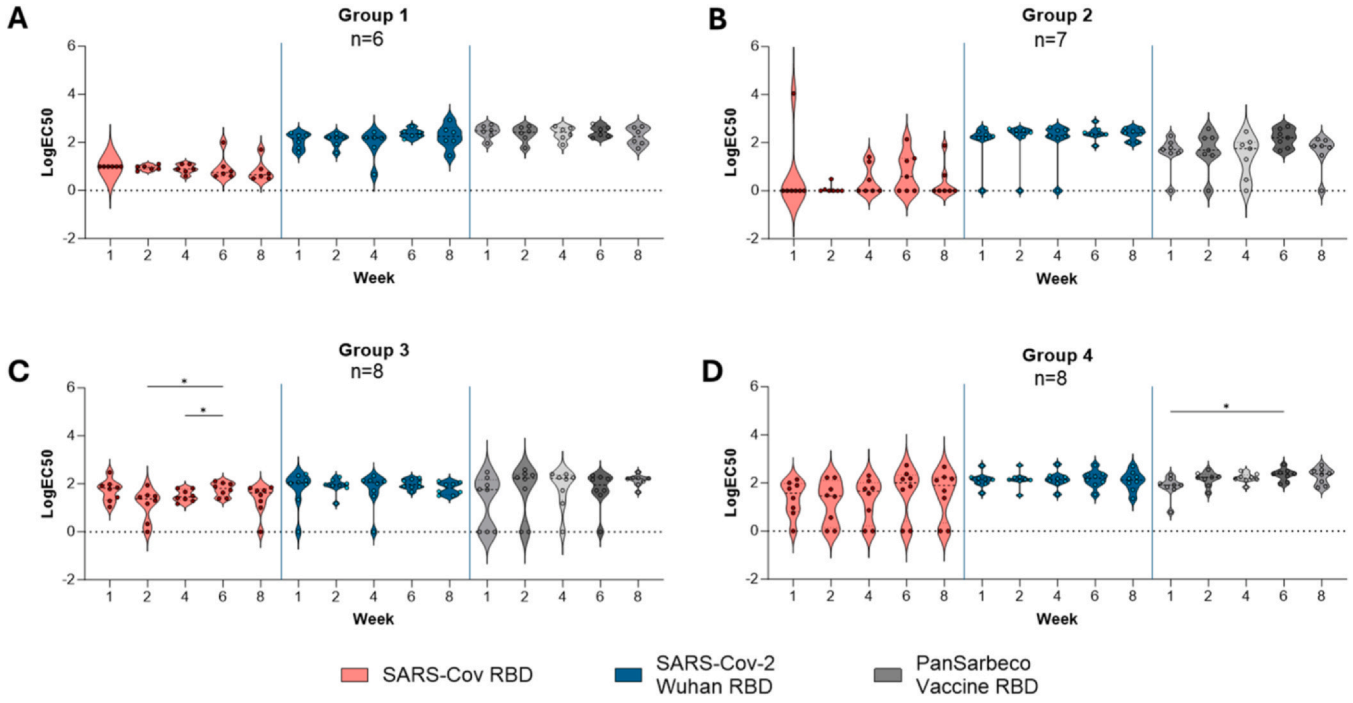

Fig. 4. Primary immunogenicity assessment. Immunogenicity assessment through enzyme-linked immunosorbent assay. ELISA binding titers are shown as $log_{10}EC_{50}$ values over time. Each dot represents the $log_{10}EC_{50}$ derived from individual serum dilution curves fitted with a four-parameter logistic (4PL) model in GraphPad Prism 10. Each violin shows the distribution of individual log_{10}-transformed EC_{50} values. The central line represents the median and the box indicates the interquartile range. The violin width represents the data distribution. Red, blue, and gray represent binding to the SARS-CoV-1 RBD, SARS-CoV-2 (Wuhan) RBD, and PanSarbeco Vaccine RBD, respectively. Sample sizes (n) for each group and antigen are indicated. Samples with fitted EC_{50} values < 1 ($log_{10}EC_{50}$ < 0) were considered below the assay's lower limit of quantification (LLOQ) and assigned a nominal $log_{10}EC_{50}$ = 0. Statistical significance across time points for each antigen was determined using the Friedman test with Dunn's post-hoc correction for multiple comparisons.

Discussion

This first in human phase I study evaluated the safety and immunogenicity of pEVAC-PS, a computationally designed pan-sarbecovirus DNA vaccine delivered intradermally without needles. The vaccine was well tolerated at all four tested doses (0.2 mg, 0.4 mg, 0.8 mg and 1. 2 mg), with no dose-dependent increase in reactogenicity. Fewer solicited adverse events recorded after the second dose, indicate good tolerability for intradermal delivery.

While safety outcomes were encouraging, the immunogenicity of pEVAC-PS was modest and variable. Binding antibody responses were observed against the vaccine construct in Group 4 (1.2 mg), but the magnitude of the response was limited and did not increase predictably with higher doses. These findings are likely influenced by heterogeneous prior SARS-CoV-2 exposure and vaccination history among participants, which confounded baseline antibody levels and complicated interpretation of boosting effects.

Neutralization activity was measured in Groups 3 and 4, with minor increases in IC50 values against SARS-CoV-2 Delta and Omicron BA.1 pseudoviruses but limited activity against the ancestral Wuhan strain and SARS-CoV-1. The magnitude of change in neutralizing titres was small, suggesting that the neutralizing potency at these dose levels may not be sufficient for broad protection. The observed discordance between ELISA binding and neutralization data likely reflects both assay sensitivity, as well as variability in prior immune priming.

One of the key design principles of the pEVAC-PS vaccine was to enhance immune responses to conserved and broadly neutralizing antibody epitopes. The peptide microarray analysis provided experimental evidence, as it characterized antibody recognition patterns and demonstrated binding to conserved regions of the RBD, including the site corresponding to the S309 epitope.[12,13] While this site has been shown in other studies to exhibit reduced in vitro neutralization against Omicron BA.2 and later variants, those same studies reported that S309 and related antibodies retain appreciable in vivo protective activity through Fc-mediated mechanisms.[12,13] Detection of antibodies targeting this conserved region in pEVAC-PS vaccine recipients therefore indicates recognition of a functionally important and cross-reactive epitope, even though such binding may not directly translate into broad functional neutralization across all Omicron sublineages. Notably, the peptide microarray analysis is biased towards linear epitopes and may underestimates recognition of conformational antibody responses that could contribute to functional neutralisation.

This trial had several important limitations. The study was conducted during evolving SARS-CoV-2 pandemic, resulting in unavoidable heterogeneity in participants infection and vaccination histories across dose groups. Whilst only a minority of participants within group one had received a third dose booster vaccine against COVID-19, this rose to all participants within group four, due to the timing of enrolment of the different groups which was done in a stepwise fashion. The exposure to circulating SARS-CoV-2 variants also differed between groups, particularly given limited or no omicron BA.1 or BA.2 exposure to group 1 prior to enrolment. There was therefore different baseline immunogenicity between groups which will have influenced both the reactogenicity and immunogenicity results.

The non-standard in-house ELISA approach and lack of a comparator vaccine limit the ability to benchmark antibody responses against known correlates of protection. Small group sizes, and narrow inclusion criteria further constrain interpretation of immunogenicity data. These factors collectively limit conclusions about dose-response relationships or pan-sarbecovirus breadth.

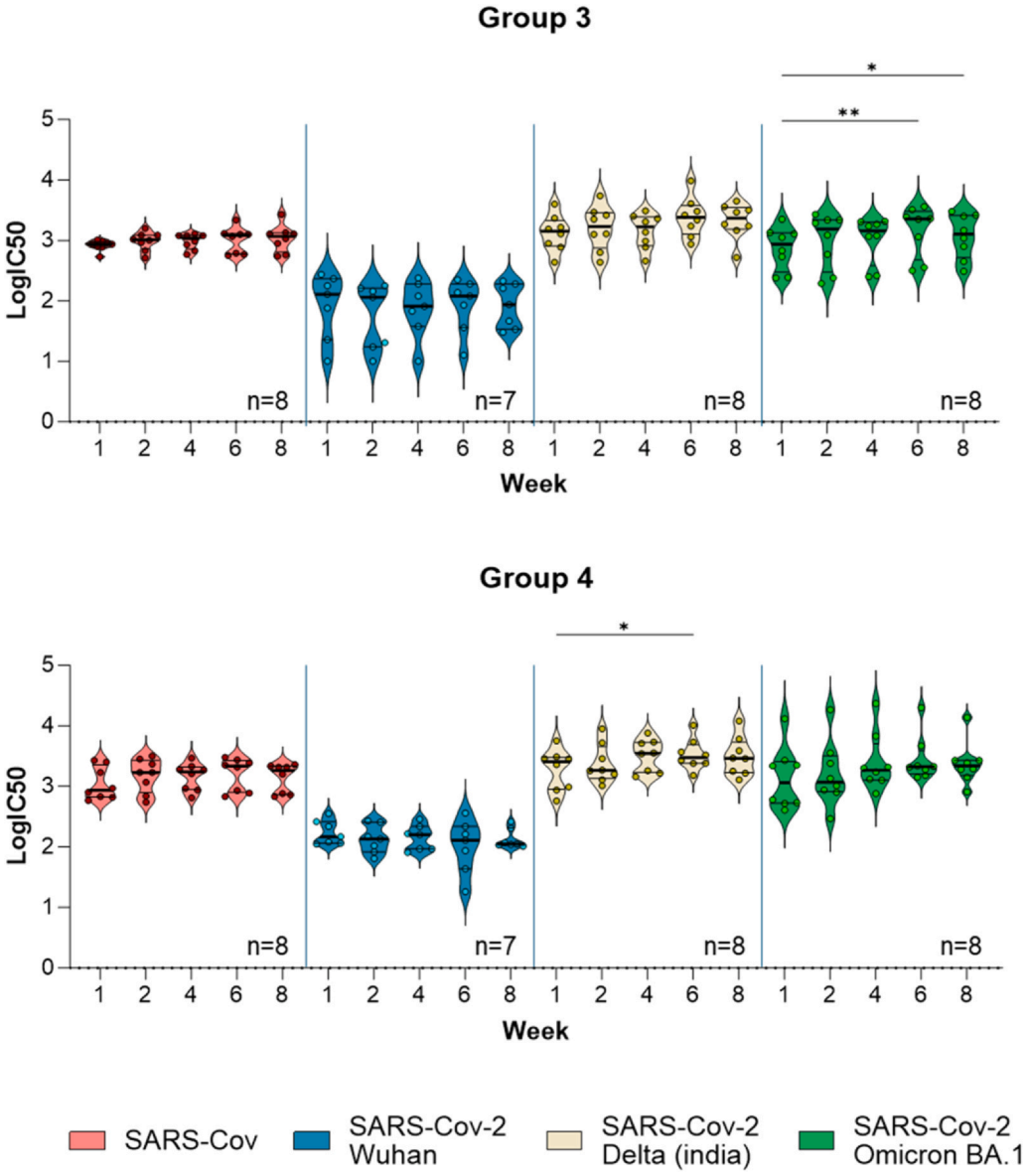

Fig. 5. Pseudovirus microneutralisation assay. The figure shows the neutralizing antibody titres (Log IC50) over time for Groups 3, and 4 against pseudoviruses representing SARS-CoV, SARS-CoV-2 Wuhan strain, SARS-CoV-2 Delta,and SARS-CoV-2 Omicron BA.1. The y-axis represents the Log10 IC50 values, indicating the neutralization potency of antibodies in each group, while the x-axis represents the time points in weeks.[1,2,4,6,8] Violin plots display the distribution of data for each group, with the width of the plot indicating the density of the data at different values. The central line represents the median and the box indicates the interquartile range. Baseline variability in neutralizing responses was observed across the four pseudoviruses. For each virus, statistical analysis was performed using the Friedman test followed by Dunn's post-hoc correction for multiple comparisons. Sample sizes (n) are indicated within the figure. Variations in n reflect the exclusion of samples with poor curve fits ($R^2 < 0.8$). Significant increases in neutralizing titers were detected in Group 3 (Omicron BA.1) and Group 4 (Delta variant) relative to baseline.

Despite these limitations, the study provides key first-in-human safety and feasibility data for DIOSynVax's computationally designed antigen platform and demonstrates the feasibility and safety of needle-free intradermal DNA vaccine delivery.

DNA vaccines remain a valuable platform for rapid antigen prototyping due to their stability, ease of manufacture, and adaptability, although their immunogenicity in humans has historically been lower than that of mRNA vaccines.[14] This difference is largely due to the additional cellular barrier DNA vaccines must overcome: plasmid DNA must penetrate the cell nucleus to be transcribed, whereas mRNA vaccines act directly in the cytoplasm, enabling faster and more robust protein expression. Despite the constraints, DNA vaccines offer key advantages, particularly in terms of storage and distribution, as they are generally more thermostable than mRNA vaccines and do not require ultra-cold chain logistics, making them well suited for use in low- and middle-income countries and in rapid-response scenarios.

Although theoretically DNA vaccines could integrate into the host genome, this risk is extremely low. Few DNA vaccines have been approved for human use (e.g., ZyCoV-D), and preclinical studies show no integration-related adverse events. Plasmid DNA is non-replicating, lacks integration machinery, leading regulatory assessments to consider the risk negligible.[15]

Needle-free intradermal administration further enhances global applicability, reducing vaccine volume requirements, avoiding sharps waste, and improving acceptance in settings where needle-based delivery is a barrier.[16]

In summary, pEVAC-PS was safe and well tolerated, with evidence of cross-reactive binding to conserved sarbecovirus epitopes. Given the ongoing risk of SARS-CoV-2 evolution and the potential for

future zoonotic coronaviruses, developing broadly protective vaccines remains a critical global health priority. While the immunogenicity outcomes of pEVAC-PS vaccine do not yet substantiate broad or robust neutralizing activity, the findings support the underlying design concept and inform further optimization of this platform for next-generation, broadly protective coronavirus vaccines.

CRediT authorship contributions statement

JLH, SNF, APSM and RK conceived the trial and SNF and JLH are the clinical and scientific chief investigators respectively. SNF, JLH, RK and APSM contributed to the protocol and design of the study. APSM led the implementation of the study alongside RB, KC and SNF. APSM and VC designed the safety analysis, and APSM conducted the safety analysis. MF designed and conducted the immunogenicity analysis. JMDR performed laboratory screening and exploratory immunogenicity experiments. APSM, JLH, MF and DE drafted the report. All other others contributed to the implementation and data collection. All authors reviewed and approved the final report.

Data availability

The study protocol and statistical analysis plan is provided in the appendix. The full anonymised data will be made available on reasonable requests made to the study Sponsor.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: SNF acts on behalf of University Hospital Southampton NHS Foundation Trust as an Investigator and/or providing consultative advice on clinical trials and studies of COVID-19 and other vaccines funded or sponsored by vaccine manufacturers including Janssen, Moderna, Pfizer, AstraZeneca, GlaxoSmithKline, Novavax, Seqirus, Sanofi, Medimmune, Merck and Valneva vaccines and antimicrobials. He receives no personal financial payment for this work. Matteo Ferrari, Sneha Vishwanath, Matthew Davies, Joanne Marie M. Del Rosario are employees of DIOSYNVAX LTD.Rebecca Kinsley, Jonathan Heeney, Ralf Wagner are shareholders of DIOSYNVAX LTD.No other competing interests are declared.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2026.106759.

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