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Safety and immunogenicity of the inactivated whole-virus adjuvanted vaccine VLA2001: a randomized, dose escalation, double-blind phase 1/2 clinical trial in healthy adults

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Abstract:	Objectives: We aimed to evaluate the safety and optimal dose of a novel inactivated whole-virus adjuvanted vaccine against SARS-CoV-2: VLA2001. Methods: We conducted an open-label, dose-escalation study followed by a double-blind randomized trial using low, medium and high doses of VLA2001 (1:1:1). The primary safety outcome was the frequency and severity of solicited local and systemic reactions within 7 days after vaccination. The primary immunogenicity outcome was the geometric mean titre of (GMT) of neutralizing antibodies against SARS-CoV-2 two weeks after the second vaccination. The study is registered as NCT0467101 7. Results: Between December 16, 2020, and June 3, 2021, 153 healthy adults aged 18-55 years were recruited in the UK. Overall, 81.7% of the participants reported a solicited AE, with injection site tenderness (58.2%) and headache (46.4%) being the most frequent. Only 2 participants reported a severe solicited event. Up to day 106, 131 (85.6%) participants had reported any AE. All observed incidents were transient and non-life threatening in nature. Immunogenicity measured at 2 weeks after completion of the two-dose priming schedule, showed significantly higher GMTs of SARS-CoV-2 neutralising antibody titres in the highest dose group (GMT 545.6; 95% CI: 428.1, 695.4) which were similar to a panel of convalescent sera (GMT 526.9; 95% CI: 336.47, 825.06). Seroconversion rates of neutralising antibodies were also significantly higher in the high-dose group (>90%) compared to the other dose groups. In the high dose group, antigen-specific interferon-γ expressing T-cells reactive against the S, M and N proteins were observed in 76, 36 and 49%, respectively. Conclusions: VLA2001 was well tolerated in all tested dose groups, and no safety signal of concern was identified. The highest dose group showed statistically significantly stronger immunogenicity with similar tolerability and safety, and was selected for phase 3 clinical development. Funding: Department of Health and Social Care, UK, Valneva Austria GmbH
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To
Robert Read
Editor-in-Chief
Journal of Infection

Vienna, May 30, 2022

Cover Letter

Dear Dr Read,

We wish to submit our manuscript entitled "Safety and immunogenicity of the inactivated whole-virus adjuvanted vaccine VLA2001: a randomized, dose escalation, double-blind phase 1/2 clinical trial in healthy adults" for consideration of publication by the *Journal of Infection*.

We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

In this paper, we report on the safety and immunogenicity of Valneva's VLA2001 vaccine, currently the only whole virus, inactivated, adjuvanted vaccine candidate in clinical trials against COVID-19 in Europe. In the phase I/II Study, we have evaluated the safety and optimal dose of VLA2001 in 153 healthy adults aged 18-55 years and found that VLA2001 was well tolerated in all tested dose groups, and no safety signal of concern was identified. The highest dose group showed significantly stronger immunogenicity with similar tolerability and safety and was thus selected for further clinical development.

The results of this study, together with the results of the pivotal phase 3 study, formed the core elements of successful regulatory submissions. On 14 of April the UK Medicines and Healthcare products Regulatory Agency has granted VLA2001 a conditional marketing authorization. In addition, on May 19, 2022, The European Medicines Agency (EMA) has accepted the filing of a marketing authorization for the VLA2001 and final CHMP opinion is expected soon. This is remarkable because VLA2001 is the first vaccine that will receive market authorization by a "gold-standard" regulatory authority based on an immunobridging approach described in the submitted article, where vaccine effectiveness is inferred through comparison of the neutralizing antibody titers to titers achieved with an already licensed vaccine with proven effectiveness.

We are therefore convinced that our manuscript is appropriate for publication by the *Journal of Infection* due to its novelty in two respects:

- as first inactivated whole-virus COVID-19 vaccine to be licensed in Europe, and
- as first COVID-19 vaccine to be licensed based on an immunobridging approach.

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We think that this novelty will be of interest to the broad readership of the *Journal of Infection* and has the potential to impact on current medical science and practice in light of the ongoing COVID-19 pandemic.

Please address all correspondence concerning this manuscript to me at Christian.taucher@valneva.com.

Thank you for your consideration of these manuscripts.

Sincerely,

Christian Taucher
(On behalf of the co-authors)

Title: *Safety and immunogenicity of the inactivated whole-virus adjuvanted vaccine VLA2001: a randomized, dose escalation, double-blind phase 1/2 clinical trial in healthy adults*

Running Title: *VLA2001*

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Safety and immunogenicity of the inactivated whole-virus adjuvanted vaccine VLA2001: a randomized, dose escalation, double-blind phase 1/2 clinical trial in healthy adults

Highlights

- VLA2001 is a whole-virus, inactivated, adjuvanted COVID-19 vaccine candidate.
- VLA2001 induces neutralizing antibodies and T-cell responses against COVID-19.
- VLA2001 is well tolerated in adults aged 18-55 years, with no safety signal of concern being identified.
- Comparison of three different dose levels allowed identification of optimum dosage for further clinical investigation.

Summary

Objectives: We aimed to evaluate the safety and optimal dose of a novel inactivated whole-virus adjuvanted vaccine against SARS-CoV-2: VLA2001.

Methods: We conducted an open-label, dose-escalation study followed by a double-blind randomized trial using low, medium and high doses of VLA2001 (1:1:1). The primary safety outcome was the frequency and severity of solicited local and systemic reactions within 7 days after vaccination. The primary immunogenicity outcome was the geometric mean titre of (GMT) of neutralizing antibodies against SARS-CoV-2 two weeks after the second vaccination. The study is registered as NCT04671017.

Results: Between December 16, 2020, and June 3, 2021, 153 healthy adults aged 18-55 years were recruited in the UK. Overall, 81.7% of the participants reported a solicited AE, with injection site tenderness (58.2%) and headache (46.4%) being the most frequent. Only 2 participants reported a severe solicited event. Up to day 106, 131 (85.6%) participants had reported any AE. All observed incidents were transient and non-life threatening in nature. Immunogenicity measured at 2 weeks after completion of the two-dose priming schedule, showed significantly higher GMTs of SARS-CoV-2 neutralising antibody titres in the highest dose group (GMT 545.6; 95% CI: 428.1, 695.4) which were similar to a panel of convalescent sera (GMT 526.9; 95% CI: 336.47, 825.06). Seroconversion rates of neutralising antibodies were also significantly higher in the high-dose group (>90%) compared to the other dose groups. In the high dose group, antigen-specific interferon-γ expressing T-cells reactive against the S, M and N proteins were observed in 76, 36 and 49%, respectively.

Conclusions: VLA2001 was well tolerated in all tested dose groups, and no safety signal of concern was identified. The highest dose group showed statistically significantly stronger immunogenicity with similar tolerability and safety, and was selected for phase 3 clinical development.

Funding: Department of Health and Social Care, UK, Valneva Austria GmbH

Keywords

Coronavirus, SARS-CoV-2, COVID-19, whole-virus vaccine, inactivated vaccine, adjuvanted vaccine, neutralizing antibody, vaccine safety, S protein binding IgG antibody, RBD-binding IgG antibody, CpG 1018, aluminum hydroxide.

Research in Context

Evidence before this study. We searched PubMed for research articles published from database inception until March 11, 2022, using the terms “COVID-19” OR “SARS-CoV-2” AND “vaccine” AND “inactivated”. Filters applied: Clinical Trial, Randomized Clinical Trial. No language and date restrictions were applied. 35 reports were identified, among which 12 described *Sinovac-CoronaVac* (Sinovac Biotech, Beijing, China), 4 *Covilo* (*BBIBP-CorV*, Sinopharm, Beijing, China), 3 *Covaxin* (*BBV152*, Bharat Biotech, Hyderabad, India), 1 *WIV04* (Sinopharm, Beijing, China), 1 *HB02* (Sinopharm, Beijing, China), 1 *KCONVAC* (Minhai Biotechnology, Beijing, China), 10 unspecified inactivated vaccines, and 4 were not relevant to our analysis. *Sinovac-CoronaVac*, *Covilo* and *Covaxin* are currently granted Emergency Use Listing by the World Health Organization (WHO); but are not authorized by the US Food and

Drug Administration [FDA], nor the European Medicines Agency [EMA]. Despite their global importance, the reported safety and the immunogenicity profile of inactivated COVID-19 vaccines is lacking.

Added value of this study: We report the safety and immunogenicity profile of the inactivated COVID-19 vaccine VLA2001. VLA2001 induces neutralizing antibodies against COVID-19 and is well tolerated. For example, fever rates, which are commonly reported after vaccination with other COVID-19 vaccines, were below 2%. It is based on a traditional and reliable manufacturing protocol utilized also for other vaccines that have received marketing approval. This type of vaccine may prove acceptable to populations that show vaccine hesitancy towards new biotechnologies (*e.g.*, mRNA, adenovirus vaccines). Manufacturing costs are low, the production is up-scalable and can be updated to new CoV variants. The vaccine can be stored at 2-8^{o}C, making it particularly suitable to be distributed around the globe.

Implications of all the available evidence: The development of safe, affordable, effective and reliable vaccines against COVID-19 that can reassure people and encourage them to get vaccinated, continues to be of great importance.

Introduction

High coverage mass inoculation against SARS CoV2, particularly when targeted efficiently at the age and risk groups at highest risk of severe disease, can impact massively on the deaths and morbidity due to COVID-19 and the consequent social and economic damage caused by the pandemic.[1] Even though several vaccines, developed using novel platforms, are currently approved by western regulatory agencies EMA and FDA (e.g., *Comirnaty*, *Spikevax*, *Vaxzevria*, *Janssen*), there are some individuals and groups that have not chosen to receive them, perhaps wary of the new biotechnologies used in their development (*i.e.*, mRNA, adenoviral vectors). Since less than a quarter of people in some low-income countries have received even a single dose of a COVID-19 vaccine (e.g. only 12% in Nigeria, 21% in Ethiopia), additional efforts to increase vaccine affordability and global access are essential - COVID-19 vaccination needs to be as inclusive as possible.[2,3]

Historically, inactivated viral vaccines, including those against polio and influenza, have been used widely and successfully worldwide and are seen as safe, reliable and effective - including in special groups such as pregnant women and immunocompromised people.[4] These inactivated inoculates contain the whole virus and, in many cases, adjuvants, inducing a broader immune response than vaccines that only include one specific viral antigen. These vaccines are readily transportable and generate few logistic problems, since they can be kept refrigerated for long periods of time. With these features in mind, *Valneva* have developed VLA2001 – an inactivated whole-virus vaccine against SARS-CoV-2 which includes a novel adjuvant.

The aim of this Phase 1/2 trial was to assess safety, reactogenicity and immunogenicity of VLA2001 in healthy adults, and to establish an optimal dose for the subsequent stages of clinical development.

Methods

Vaccine manufacture.

VLA2001 uses a viral strain derived from a Chinese tourist from Hubei, diagnosed in a hospital in Rome[5], and an inactivated whole-virus approach in which live wild-type SARS-CoV-2 virus is grown in cultured Vero cells. After virus propagation, β-propiolactone is used for viral inactivation in order to preserve the native surface structure of the virion, in a robust process that yields high-density and intact Spike protein.[6] Additionally, VLA2001 is adjuvanted with cytosine phospho-guanine (CpG) 1018 and aluminum hydroxide.

Study design and participants.

This study is an open label, dose-escalation trial in groups of 5, followed by a double-blind randomized trial of low-, medium- and high-doses of VLA2001 in 3 planned groups of 45 subjects with a 1:1:1 allocation for the three specific dose regimes. Written informed consent was received from all participants and the trial was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. The study was approved in the UK by the Medicines and Healthcare Products Regulatory Agency (43185/0002/001-0001) and the London, Brent ethics committee ref 20/HRA/5205.

Key exclusion criteria were acute illness, pregnancy, known prior SARS-CoV-2 infection, and any immunosuppressive condition or receipt of immunosuppressive therapy. Full eligibility criteria are listed in the protocol (**Appendix 1**). The study participants were enrolled from 4 study sites across the UK. The investigators at each hospital were responsible for the conduct of the study as per approved protocol and were blinded for study assignments.

Dose escalation was done at a single site to ensure permanent oversight on safety data by one Principal Investigator. A Data Safety and Monitoring Board (DSMB) reviewed the accrued safety data at day 4 of all 15 sentinel participants. After favorable DSMB review, recruitment of the remaining participants (eventually 138 randomised) across all sites was initiated.

Randomization and blinding.

In the blinded trial, the participants were randomized in a 1:1:1 fashion to one of the dose levels of VLA2001: low-, medium-, or high-dose. The randomization code was generated by the statistician, and allocation by the trial team was done via interactive web response system assigned at the screening visit. The investigational medicinal product (IMP) was provided to the trial sites in identical packaging for all strengths of VLA2001. IMP allocation was based on an identifier linked to the randomization; and, since there was no visual difference between the doses, all trial staff and participants remained blinded to dose allocation. The randomization assignment was not to be revealed except in emergency cases where unblinding was necessary for clinical management.

Procedures.

The vaccination schedule consisted of two vaccinations for each study participant 21 days apart, administered by intra-muscular (IM) injection into the deltoid muscle of the non-dominant arm. VLA2001 is an inactivated, SARS-CoV-2 vaccine adjuvanted with CpG 1018 and aluminum hydroxide (0.5mg/dose). The antigen units (AU) of the different VLA2001

dosages were evaluated and confirmed in the final product using Spike-protein ELISA: 3 AU (low-dose), 7AU (medium-dose) or 35AU (high-dose). The active substance was combined with CpG 1018 (1mg/dose) to reach the final concentrations in 0.5mL immediately before administration.

At baseline (visit 0), participants were physically examined and medical history was sought for any pre-existing conditions. At visit 1, prior to vaccination, any abnormal conditions were recorded and at all other study visits, any new abnormal or worsened pre-existing conditions were recorded as adverse events (AE).

To ensure safety, the first 15 participants were included into the study in an open-label, non-randomized manner following a staggered dose escalation of VLA2001. First, a single sentinel participant in the low dose treatment group received their first vaccination. After vaccination, the first participant of each dosing group was observed for the development of any acute reaction at the study site for 3 hours after the vaccination procedure. The study site contacted the participant by phone ≈24 hours after vaccination to assess their safety status. The subsequent 4 participants of each successive dosing group were vaccinated with a minimum 1 hour interval between each and likewise observed. After confirmation by the Investigator that no stopping criteria had been met, the study proceeded to the next dose level. The minimum observation period before initiation of vaccination at a new dose level was 48 hours.

In the blinded randomized trial of the remaining participants, vaccinations were administered on days 1 and 22. Participants were asked to complete a eDiary daily for 7 days following each vaccination in which solicited local (injection-site pain, tenderness, redness, itching, swelling, and induration) and/or systemic (fever/body temperature, fatigue, headache, myalgia and nausea/vomiting) AEs were recorded throughout the study. Additionally, serious AEs (including all cases of COVID-19, as well as immune-mediated disorders that might be caused by the adjuvant CpG 1018) were collected. Investigators followed all AEs and assessed likelihood of any causal relationship with study vaccines based on clinical judgement. Participants who developed any potentially COVID-19-related symptoms after randomization were requested to attend for PCR testing for SARS-CoV-2 without delay if they had high fever (≥38.0°C or ≥100.4°F) or shortness of breath or, after two consecutive days, if symptoms were milder (*e.g.*, sore throat, chills, cough, body aches, new loss of taste or smell, runny nose, nausea, or diarrhea).

Venous blood samples were taken on days 1, 8, 22, 36 and 106. Antibody responses at these time points were measured using an immunoassay for IgG to full length Spike-protein (S-protein, Nexelis, Canada), and a live microneutralization assay MNA_{50} against the Victoria strain performed by Public Health England (Porton Down, UK; now UK Health Security Agency and Office for Health Improvement and Disparities)[7]. Geometric mean titres (GMT) of neutralizing antibody were also compared to a published panel of COVID-19 convalescent sera[7]. Cellular immunity against Spike-protein (S-protein), Nucleocapsid-protein (N-protein) and Membrane-protein (M-protein) were assessed at Oxford Immunotec using T-Spot Discovery SARS-CoV-2 (Oxford, UK). T-cell responses were classified as reactive if 6 or more Spot Forming Units (SFU) per $2x10^5$ Peripheral Blood Mononuclear Cells (PBMC) were present, upon subtraction of control cell counts.

Outcomes.
The primary safety outcome was the frequency and severity of solicited local and systemic reactions within 7 days after vaccination. Secondary safety outcomes were the frequency and severity of any adverse event throughout the study, including serious adverse events (SAEs). The primary immunogenicity outcome was the geometric mean titre (GMT) of neutralizing antibodies against SARS-CoV-2 at day 36 (2 weeks after the second vaccination). GMT of IgG SARS-CoV-2 S-protein binding antibodies as well as seroconversion in terms of neutralizing and S-binding antibodies were secondary outcomes. GMT and seroconversion rates were determined at days 1, 8, 22, 36 and 106.
As an exploratory outcome, T-cell immune responses are also described.

Statistical Analysis.
This was a descriptive trial and formal power calculations were not performed. It was agreed with regulators in advance that a total of 150 participants would be sufficient for initial safety evaluation, allowing for 95% confidence that an AE with a true underlying incidence of about 2% would be observed. The safety analysis included all participants who received at least a single dose of vaccine. The immunogenicity analysis was performed on the per-protocol population, which excluded any participants who received less than two vaccinations, received the wrong trial medication or fulfilled pre-defined exclusion criteria. The results for all participants were combined for analysis and reporting including those enrolled in an open label, non-randomized manner. Differences between treatment groups relating to AEs were assessed for significance using Fisher's exact test. The number and percentage of subjects with solicited injection site and systemic AEs within 7 days after vaccination along with the exact 95% Clopper-Pearson confidence interval (CI) for all AE rates were presented for each dose group and overall. Differences between the dose groups were assessed for significance using the Fisher-Freeman-Halton exact test and p-values are presented for this test. GMTs (CI) were calculated by taking the antilogarithm of the mean (CI) of the log10 transformed titres. P-values were also calculated using the Kruskal Wallis test to check whether the results were significantly different among dose groups at 5% level of significance. If the test suggested significance, then a pairwise group comparison was performed using the Dwass, Steel, Critchlow-Fligner (DSCF) multiple comparisons post-hoc procedure to determine which pair of dose groups differed significantly. Secondary immunogenicity analyses included comparison of the seroconversion rates (SCRs) on days 22, 36 and 106 using the Fisher-Freeman-Halton exact test. If the overall difference between groups was statistically significant (i.e., p-value for Fisher-Freeman-Halton exact test was ≤ 0.05), then multiplicity adjusted p-values (using Hochberg method) for pairwise group differences were calculated using Fisher's exact test. Seroconversion was defined as at least a four-fold increase in GMT from baseline. Statistical analyses using SAS® version 9.4 was performed by Valneva and were independently verified by LD using R version 4.0.2.

Role of the funding source.
The funder Valneva GmbH designed the study and performed the data analyses that led to this manuscript.

Results

285 individuals aged between 18 and 55 years were screened and 153 were enrolled in the study, with 51 participants in each dose group (Figure 1). The statistical analysis described herein was performed after all participants had reached the day 106 visit after first vaccination. There were no participants who terminated early before day 106. 143 individuals were included in the Per Protocol (PP) population for immunogenicity analysis, all 153 participants were included in the safety analysis. The mean age of participants was 33.5 years, 54% were male and 93.5% were white, with the remaining 6.5% of Asian, Mixed and Latin-American ethnic origin (Table 1).

Safety and Dosage As described in Table 2, up to day 106, 85.6% of participants reported an AE. Overall AE incidence rates were 88.2% in the low-dose, 78.4% in the medium-dose and 90.2% in the high-dose groups. Solicited AEs were reported in 81.7% of participants. 66.7% of the participants reported at least one solicited injection site reaction within 7 days after any vaccination. Injection site tenderness was the most commonly reported solicited AE (58.2%), followed by pain (41.8%), itching (5.2%) and swelling (1.3%) at the injection site. While the incidence of injection site tenderness in the medium-dose group was lower (45.1%) than in the low- and high-dose groups (62.7% and 66.7%, respectively), there were no statistically significant differences among the treatment groups (Table S1). Overall, injection site reactions were mild, and there were no severe or potentially life-threatening events, whether after the first or second vaccination (Table S2).

Overall, 68.6% of the participants reported a solicited systemic reaction within 7 days after any vaccination. Headache was reported most frequently (46.4%), followed by fatigue (39.2%), muscle pain (32.0%), nausea/vomiting (11.8%), and fever/body temperature (1.3%) (Table S 3). While the incidence of headache in the medium-dose group was lower (33.3%) than the low- and high-dose groups (54.9% and 51.0%, respectively), there was no statistically significant difference between the treatment groups. The incidence of fatigue in the medium-dose group was lower (29.4%) than in the low- and high-dose groups (45.1% and 43.1%, respectively), but again with no statistically significant difference across the treatment groups. The incidences of muscle pain, nausea/vomiting and fever/body temperature were similar and comparable following the first or second vaccination (Table S4).

Up to the day 106 data-cut-date, a total of 43.8% participants reported an unsolicited AE (Table S5). The incidences of unsolicited AEs were 49.0%, 37.3%, and 45.1%, respectively, in the low-, medium- and and high-dose groups. Most unsolicited AEs occurred up to day 36: Overall, 41.8% of participants reported at least one unsolicited AE up to day 36, 47.1%, 35.3%, and 43.1% %, respectively, in the low-, medium- and and high-dose groups.

The incidences of any medically attended unsolicited AEs until day 36 were 5.9% in the medium-dose group and 2.0% in both the low- and high-dose groups. Until day 106, 5.9% in the low- and high-dose groups (and 11.8% in the medium-dose group reported medically attended unsolicited adverse events(Table S5). By day 106, increased red blood cell sedimentation rate (9.2%) was the only unsolicited AE occurring in >5% of participants. The incidence was 9.8% in the low- and high-dose groups and 7.8% in the medium-dose group (Table S6).

The vast majority of AEs reported in the study were mild or moderate. Only two participants reported severe solicited systemic AEs (Table S7); there were no severe vaccine-related unsolicited events and no serious treatment-related adverse events.
One AESI was reported which was a mild case of chilblains 4 days after first vaccination. The participant tested negative for COVID-19, had normal platelets and the event was considered unrelated to vaccination and a second vaccine dose was administered without further event. As per protocol definition, any AESI was treated as serious adverse event in this study. No other AESI nor SAE was reported up to day 106.

Number of COVID-19 Infections: There were three confirmed mild and moderate COVID-19 infections detected, two in the low- and one in the medium-dose groups (Table S 8).

Immunogenicity, SARS-CoV-2 Neutralizing Antibody Titers: Only a slight increase of GMT was observed by day 22 (36.8, 39.9 and 47.7 in the low dose, medium dose, and high dose groups respectively); by day 36, GMTs ranged from 168.7 (95% CI: 125.1, 227.5) in the low dose group to 545.6 (95% CI: 428.1, 695.4) in the high dose group. Statistically significant differences ($p<0.001$) were seen amongst the 3 dose groups (Table 3 and Figure 2). Pairwise comparison indicated statistically significant differences between both the low- and medium-dose groups *vs*. the high-dose group ($p<0.001$), with a clear dose dependent response and with a peak titre at day 36 (Table 3).
Of note, at day 36, the GMT of neutralising antibody titres in the high dose-group was similar to those measured in a panel of COVID-19 convalescent sera (GMT 526.9 [95% CI: 336.47, 825.06) (Table 3). The data presented consist of a panel of 32 serum samples which have been described previously as part of a larger panel of sera.[7] Based on the Kruskal Wallis Test, a statistically significant difference ($p<0.001$) was seen in an overall comparison across all dose groups and the convalescent serum panel, indicating that the GMTs were not the same across the four groups at day 36. The GMT at day 36 of the high dose group was significantly higher than of both the medium and low dose groups ($p<0.001$) and similar to the GMT of the panel of convalescent sera ($p>0.999$).
By day 106, GMT ranged from 63.3 in the low-dose group to 175.9 in the high-dose group. Statistically significant differences ($p<0.001$) were seen between the 3 dose groups. and comparing the low- and medium-dose groups with the high-dose group ($p=0.001$ and $p<0.001$, respectively).

Seroconversion. The seroconversion rate between day 1 and day 36 following the second vaccine dose was 91.1% in the high dose group with statistically significantly lower rates in the medium and low dose groups ($p<0.001$; 71.4% and 50.0%, respectively) (Table 4). The day 106 seroconversion rate in the high dose group was 60.0%, again with statistically significantly lower rates in the medium and low dose groups ($p<0.001$)(Table 4).

IgG Antibody Titers against SARS-CoV-2 S-protein (ELISA). Results of immunoassays for serum anti-S binding antibodies were concordant with neutralizing antibody results. Detectable rises in antibody were seen in only a small minority of subjects in all 3 dose groups after the first vaccine dose, but in the overwhelming majority after the second dose on day 36 (Figure 3

and Table 5). Again, the GMTs were substantially and statistically significantly higher in the high dose group than in the other two groups at this time point ($p<0.001$) (Table 5).

Correlation between neutralizing antibody titers and IgG antibody titers. As an exploratory analysis, correlation coefficients were calculated between neutralizing antibody titers (ND50) and IgG antibody titers (ELISA) at the different time-points. The overall correlation coefficient between ELISA (ELU/mL) and MNA (ND50) was 0.845 ($p <0.001$) while the correlation was much stronger in the days 36 and 106 sera when the large majority of subjects had made detectable responses than at the earlier pre- and post one dose vaccination time points (Figure 4).

T-cell responses measured by IFN-γ T-cell ELISpot (T-spot): In terms of T-cell responses by IFN-γ with the T-cell ELISpot (T-spot) assay, several panels were tested in all study groups. In the high dose group, a reactive response was observed in 75% (34/45 participants) against S-protein, 36% (16/45) against M-protein and 49% (22/45) against N-protein. Figure 5 shows box plots of IFN-gamma spot forming units for spike, nucleocapsid and membrane protein panels. Statistically significant differences were seen bewteen the dose groups at day 36 for all panels.

Discussion

Here, we report interim safety, tolerability, and immunogenicity results of a phase 1 safety and dose-finding vaccine trial with an initial open label sentinel dose-escalation phase, followed by a double-blind randomized phase for the whole-virus inactivated adjuvanted COVID-19 vaccine, VLA2001. The vaccine was well tolerated, and no safety signals of concern were detected. The observed solicited adverse events such as injection site tenderness, pain, headache were mostly mild with rates similar to those commonly seen with other inactivated vaccines while fever was hardly ever reported following vaccination. All of the incidents observed were transient in nature and no serious adverse events considered related to treatment were reported. These results suggest the reactogenicity profile of this vaccine at the doses tested is likely to be favourable and support progression to further studies in order to create a larger and more robust safety database.

The immunogenicity results of this trial show a dose-dependent rise in both neutralizing and binding antibody titres which occurred predominantly after the second vaccine dose and which was substantially greater in the high dose group than in the two lower dose groups, reaching functional antibody titres that were very similar to those seen in a panel of early COVID19 convalescent sera[7]. These data encourage us to speculate that this formulation, used at the higher dose tested, may be efficacious against SARS CoV2 infection and disease and, given its low reactogenicity, may prove to be a clinically useful tool.

Peak antibody titres were reached 2 weeks after the second dose of vaccine, with 90% of those in the high dose group achieving seroconversion with regards to neutralising antibody titres. In contrast, 3 weeks after the first dose of vaccine only 10% of participants had successfully seroconverted and this modest response after one dose of VLA2001 is also reflected in the

GMTs of both neutralisation and binding antibody after that dose. This suggests that both doses of the vaccine may be needed for an adequate immune response, and it is not clear how this would translate into efficacy, if any, after only a single dose. High levels of effectiveness have been reported after single doses of other approved vaccines that were designed to be given as a 2-dose priming regimen.[10] Our results may reflect the relatively early time point at which immunogenicity was initially evaluated. Vaccine induced antibody concentrations usually peak about 4 weeks after immunisation, although this may vary between different vaccine types and longer dose intervals may improve the immunogenicity of two dose regimens as has been demonstrated for other COVID-19 vaccines.[11,12]

In addition to the humoral immunogenicity, VLA2001 also induced detectable specific T-cell responses against the S-protein M-protein and N-protein in many subjects. Few data on T-cell responses have been published for the aluminum adjuvanted inactivated COVID-19 vaccines to date.[4] Zhang et al, reported poor T-cell response measured by ELISPOT, although only S-protein specific responses were reported.[13] In the Phase 1 study of BBV152, another whole virion, inactivated vaccine against COVID-19 formulated with a Toll like receptor 7/8 agonist, T-cell ELISPOTs performed on a small number of participants demonstrated an increase in antigen specific IFN-γ secreting CD4+ T-cells in vaccinated participants, suggestive of a Th1 response.[14] The induction of a broad T-cell response by VLA2001 described in this study may be due to a higher antigen content compared to other inactivated vaccines or the presence of the CpG1018 adjuvant, which was shown to enhance immunogenicity in preclinical studies of VLA2001. Cellular immunity is considered to be important for protection against natural infection, so that an effective COVID-19 vaccine would ideally need to induce both cellular and humoral immunity while avoiding generation of potentially harmful Th2 responses.[15–19]

The main limitation of this study is that is has been performed on younger, predominantly white, adults, therefore there are no data on older adults and other ethnic groups who have a greater risk of severe COVID-19 infection. Only one dosing interval was evaluated therefore it may be that longer dosing intervals would result in improved immunogenicity.

In conclusion, VLA2001 was highly immunogenic with more than 90% of all study participants developing significant levels of antibodies to the SARS-CoV-2 virus spike protein across all dose groups tested. VLA2001 induced a dose dependent response with statistically significantly higher GMTs for both IgG and neutralising antibodies in the high dose group compared to the low and medium dose groups. The high dose group (35AU) of VLA2001 was selected for further Phase 3 development on the basis of a comparable safety profile and superior immunogenicity as compared to the low and medium dose groups enrolled in study VLA2001-201.

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Contributors

CT conceptualized and supervised the study. AF is the chief investigator. RL, CG, CJAD and SNF are study site principal investigator. SEL and JCJ contributed to the protocol and design

of the study. BQ contributed to the implementation of the study. AM, APSM, ARR ,ASG, CB, DP, DK, FP, HW, IC, JKW, KG, KN, LD, RH, SW, and TR contributed to data collection. CT, RL, and AF wrote the manuscript. SEL, JCJ, and KD reviewed and edited the manuscript. The Valneva Phase 1 study group (see table at the end of the paper) are study site principal investigators. All authors reviewed and approved the final version. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interest

AF is a member of the Joint Committee on Vaccination and Immunisation and chair of the WHO European Technical Advisory Group of Experts on Immunisation. He is an investigator or provides consultative advice on clinical trials and studies of COVID-19 vaccines produced by AstraZeneca, Janssen, Valneva, Pfizer, and Sanofi, and of other vaccines from these and other manufacturers, including GlaxoSmithKline, VPI Pharmaceuticals, Takeda, and Bionet Asia. He receives no personal remuneration or benefits for any of this work.
CTA, KD, SEL, RH, IC, BQ, and JCJ are employees of Valneva Austria GmbH.

Data sharing

Anonymized individual participant data will be made available when the study is complete, on reasonable requests made to the corresponding author. Proposals will be reviewed and approved by the sponsor, investigator, and collaborators based on scientific merit. After approval of a proposal, data can be shared through a secure online platform. All data will be made available for a minimum of 5 years from the end of the trial.

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The views expressed in this publication are those of the author(s) and not necessarily those of their employers or funders.

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Tables

Table 1 - Demographic Characteristics (Safety Population)				
Characteristics	**Low Dose N=51**	**Medium Dose N=51**	**High Dose N=51**	**Overall N=153**
Age at time of informed consent (years)				
n	51	51	51	153
Mean (SD)	33.7 (8.89)	35.5 (9.53)	31.3 (8.52)	33.5 (9.10)
Median	31.0	33.0	29.0	32.0
Min, max	21, 53	21, 55	18, 54	18, 55
Sex, n (%)				
Male	27 (52.9)	34 (66.7)	22 (43.1)	83 (54.2)
Female	24 (47.1)	17 (33.3)	29 (56.9)	70 (45.8)
Diverse	0	0	0	0
Childbearing potential				
n	51	51	51	153
Yes	23 (45.1)	16 (31.4)	28 (54.9)	67 (43.8)
No	1 (2.0)	1 (2.0)	1 (2.0)	3 (2.0)
Reason for no childbearing potential				
Surgically sterile	1 (2.0)	0	0	1 (0.7)
Postmenopausal	0	1 (2.0)	1 (2.0)	2 (1.3)
Ethnicity, n (%)				
White	46 (90.2)	51 (100.0)	46 (90.2)	143 (93.5)
Black	0	0	0	0
Asian	1 (2.0)	0	0	1 (0.7)
Mixed	0	0	4 (7.8)	4 (2.6)
Latin [A]	1 (2.0)	0	0	1 (0.7)
Latin American [A]	2 (3.9)	0	0	2 (1.3)
Latino [A]	1 (2.0)	0	0	1 (0.7)
White European [B]	0	0	1 (2.0)	1 (0.7)
Body Mass Index (BMI) at screening (kg/m^2)				
n	51	51	51	153
Mean (SD)	24.87 (2.962)	25.06 (2.238)	24.5 (3.085)	24.81 (2.778)
Median	24.7	24.9	24.5	24.8
Min, Max	19.6, 29.9	20.2, 29.2	18.1, 29.8	18.1, 29.9
COVID-19 test result at screening				
Positive	0	0	0	0
Negative	51 (100.0)	51 (100.0)	51 (100.0)	153 (100.0)
Hepatitis-BsAg test result at screening				
Positive	0	0	0	0

Negative	51 (100.0)	51 (100.0)	51 (100.0)	153 (100.0)
HCV test result at screening				
Positive	0	0	0	0
Negative	51 (100.0)	51 (100.0)	51 (100.0)	153 (100.0)
HIV test result at screening				
Positive	0	0	0	0
Negative	51 (100.0)	51 (100.0)	51 (100.0)	153 (100.0)

Max=maximum; Min=minimum; SD=standard deviation
A: Latin / Latino American / Latino: confirmed all 4 participants as Latin American with Mexico as country of birth.
B: White European

Table 2 - Overall Summary of Adverse Events (Safety Population)				
Number of	**Low Dose N=51 n (%)**	**Medium Dose N=51 n (%)**	**High Dose N=51 n (%)**	**Overall N=153 n (%)**
Participants with any AE until Day 36	45 (88.2)	40 (78.4)	45 (88.2)	130 (85.0)
Participants with any AE until day of data cut for Day 106 analysis	45 (88.2)	40 (78.4)	46 (90.2)	131 (85.6)
Participants with any treatment related AE until Day 36	43 (84.3)	40 (78.4)	43 (84.3)	126 (82.4)
Participants with any treatment related AE until day of data cut for Day 106 analysis	43 (84.3)	40 (78.4)	43 (84.3)	126 (82.4)
Participants with any serious AE until Day 36	0	1 (2.0)	0	1 (0.7)
Participants with any serious AE until day of data cut for Day 106 analysis	0	1 (2.0)	0	1 (0.7)
Participants with any medically attended AE until Day 36	1 (2.0)	3 (5.9)	1 (2.0)	5 (3.3)
Participants with any medically attended AE until day of data cut for Day 106 analysis	3 (5.9)	6 (11.8)	3 (5.9)	12 (7.8)
Participants with any solicited event	43 (84.3)	39 (76.5)	43 (84.3)	125 (81.7)

AE=Adverse Event

Table 3 - SARS-CoV-2 Neutralizing Antibody Titres (ND50) Over Timepoints – Day 1, Day 36, and Day 106 (Per Protocol Population)

Visit Statistic	Low Dose N=50	Medium Dose N=49	High Dose N=45	Overall N=144	Convalescent Subjects (N=32)
Day 1					
n	50	49	45	144	–
GMT (95% CI)	31.0 (31.00, 31.00)	32.8 (30.48, 35.24)	31.5 (30.66, 32.37)	31.8 (30.94, 32.58)	–
Median	31.0	31.0	31.0	31.0	–
Min, Max	31.0, 31.0	31.0, 232.0	31.0, 64.0	31.0, 232.0	–
p-value: overall dose groups comparison [A]	–	–	–	0.366	–
Day 36					
n	50	49	45	144	32
GMT (95% CI)	168.7 (125.09, 227.48)	218.9 (169.41, 282.92)	545.6 (428.10, 695.37)	266.0 (225.10, 314.39)	526.9 (336.47, 825.06)
Median	122.5	233.0	537.0	264.5	606.0
Min, Max	31.0, 3618.0	31.0, 1307.0	31.0, 2033.0	31.0, 3618.0	31.0, 6704.0
p-value: overall dose groups comparison [A]	–	–	–	<0.001	–
p-value: low dose vs medium dose [B]	–	–	–	0.358	–
p-value: medium dose vs high dose [B]	–	–	–	<0.001	–
p-value: low dose vs high dose [B]	–	–	–	<0.001	–
p-value: low dose vs convalescent [B]	–	–	–	0.001	–
p-value : medium dose vs convalescent [B]	–	–	–	0.023	–
p-value: high dose vs convalescent [B]	–	–	–	>0.999	–
Day 106					
n	49	49	45	143	–
GMT (95% CI)	63.3 (50.42, 79.48)	82.4 (64.26, 105.63)	175.9 (136.02, 227.56)	95.6 (82.23, 111.08)	–
Median	31.0	77.0	211.0	89.0	–
Min, Max	31.0, 1088.0	31.0, 1589.0	31.0, 1357.0	31.0, 1589.0	–
p-value: overall dose groups comparison [A]	–	–	–	<0.001	–
p-value: low dose vs medium dose [B]	–	–	–	0.366	–
p-value: medium dose vs high dose [B]	–	–	–	0.001	–
p-value: low dose vs high dose [B]	–	–	–	<0.001	–

CI=confidence interval; DSCF= Dwass, Steel, Critchlow-Fligner; GMT=geometric mean titre; Max=maximum; Min=minimum; ND50=50% neutralizing dilution
A: p-value was calculated using Kruskal Wallis Test for comparison of dose groups.
B: p-value for pairwise dose group comparison was calculated using DSCF multiple comparisons post-hoc procedure. This was calculated only if the Kruskal Wallis test was significant (i.e., p-value for overall dose groups comparison was ≤0.05.).

Table 4 - Proportion of Participants with Seroconversion in Terms of Neutralizing antibodies (ND50) at Day 36 and Day 106 (Per Protocol Population)				
Visit	**Low Dose N=50**	**Medium Dose N=49**	**High Dose N=45**	**Overall N=144**
Day 36				
Participants with seroconversion (≥4-fold increase)				
n (%)	25 (50.0)	35 (71.4)	41 (91..1)	101 (70.1)
95% CI [A]	0.36, 0.64	0.57, 0.83	0.79, 0.98	0.62, 0.77
p-value [B]	–	–	–	<0.001
p-value: low dose vs medium dose [C]	–	–	–	0.040
p-value: medium dose vs high dose [C]	–	–	–	0.038
p-value: low dose vs high dose [C]	–	–	–	<0.001
Participants with				
≥2-fold increase, n (%)	43 (86.0)	44 (89.8)	44 (97.8)	131 (91.0)
≥10-fold increase, n (%)	13 (26.0)	17 (34.7)	34 (75.6)	64 (44.4)
≥20-fold increase, n (%)	8 (16.0)	8 (16.3)	21 (46.7)	37 (25.7)
Day 106				
Participants with seroconversion (≥4-fold increase)				
n (%)	11 (22.0)	14 (28.6)	27 (60.0)	52 (36.1)
95% CI [A]	0.12, 0.36	0.17, 0.43	0.44, 0.74	0.28, 0.45
p-value [B]	–	–	–	<0.001
p-value: low dose vs medium dose [C]	–	–	–	0.495
p-value: medium dose vs high dose [C]	–	–	–	0.007
p-value: low dose vs high dose [C]	–	–	–	0.001
Participants with				
≥2-fold increase, n (%)	22 (44.0)	29 (59.2)	38 (84.4)	89 (61.8)
≥10-fold increase, n (%)	4 (8.0)	5 (10.2)	15 (33.3)	24 (16.7)
≥20-fold increase, n (%)	2 (4.0)	1 (2.0)	4 (8.9)	7 (4.9)

CI=confidence interval; ND50=50% neutralizing dilution

Note: Seroconversion was defined as ≥4-fold increase in SARS-CoV-2-specific neutralizing antibody titre levels between Day 1 and post-vaccination sample collection timepoints.

A: Exact 95% Clopper-Person CI for proportion.

B: Fisher-Freeman-Halton exact test for overall dose group differences.

C: Multiplicity adjusted p-values (using Hochberg method) for pairwise group differences from Fisher's exact test if the overall group difference was statistically significant (i.e., p-value for Fisher-Freeman-Halton exact test is ≤0.05).

Table 5 - IgG Antibody Titres Against SARS-CoV-2 S-protein (ELISA) at Day 1, 36 and 106 (Per Protocol population)

Visit Statistic	Low Dose N=50	Medium Dose N=49	High Dose N=45	Overall N=144
Day 1				
n	50	49	45	144
GMT (95% CI)	25.6 (24.58, 26.73)	26.4 (24..07, 29.05)	25.0 (25.00, 25.00)	25.7 (24.83, 26.61)
Median	25.0	25.0	25.0	25.0
Min, Max	25.0, 87.2	25.0, 390.8	25.0, 25.0	25.0, 390.8
p-value: overall dose groups comparison [A]	–	–	–	0.632
Day 36				
n	50	49	45	144
GMT (95% CI)	329.7 (247.81, 438.73)	691.6 (494.91, 966.52)	2116.0 (1642.59, 2725.75)	758.4 (622.70, 923.79)
Median	300.4	780.1	2087.7	778.4
Min, Max	25.0, 5566.1	25.0, 8637.6	116.8, 15419.5	25.0, 15419.5
p-value: overall dose groups comparison [A]	–	–	–	<0.001
p-value: low dose vs medium dose [B]	–	–	–	0.005
p-value: medium dose vs high dose [B]	–	–	–	<0.001
p-value: low dose vs high dose [B]	–	–	–	<0.001
Day 106				
n	49	49	45	143
GMT (95% CI)	111.4 (81.26, 152.68)	201.5 (151.36, 268.31)	524.8 (407.38, 675.99)	222.3 (184.72, 267.46)
Median	89.1	254.8	545.9	236.1
Min, Max	25.0, 5499.7	25.0, 2409.7	58.4, 6601.7	25.0, 6601.7
p-value: overall dose groups comparison [A]	–	–	–	<0.001
p-value: low dose vs medium dose [B]	–	–	–	0.016
p-value: medium dose vs high dose [B]	–	–	–	<0.001
p-value: low dose vs high dose [B]	–	–	–	<0.001

CI=confidence interval; DSCF= Dwass, Steel, Critchlow-Fligner; ELISA=enzyme-linked immunosorbent assay; gG=immunoglobulin gamma; GMT=geometric mean titre; Max=maximum; Min=minimum

A: p-value was calculated using Kruskal Wallis Test for comparison of dose groups.

B: p-value for pairwise dose group comparison was calculated using DSCF multiple comparisons post-hoc procedure. This was calculated only if the Kruskal Wallis test was significant (i.e., p-value for overall dose groups comparison was ≤0.05).

Figures

Figure 1 – CONSORT Flow Chart VLA2001 Phase 1/2 trial

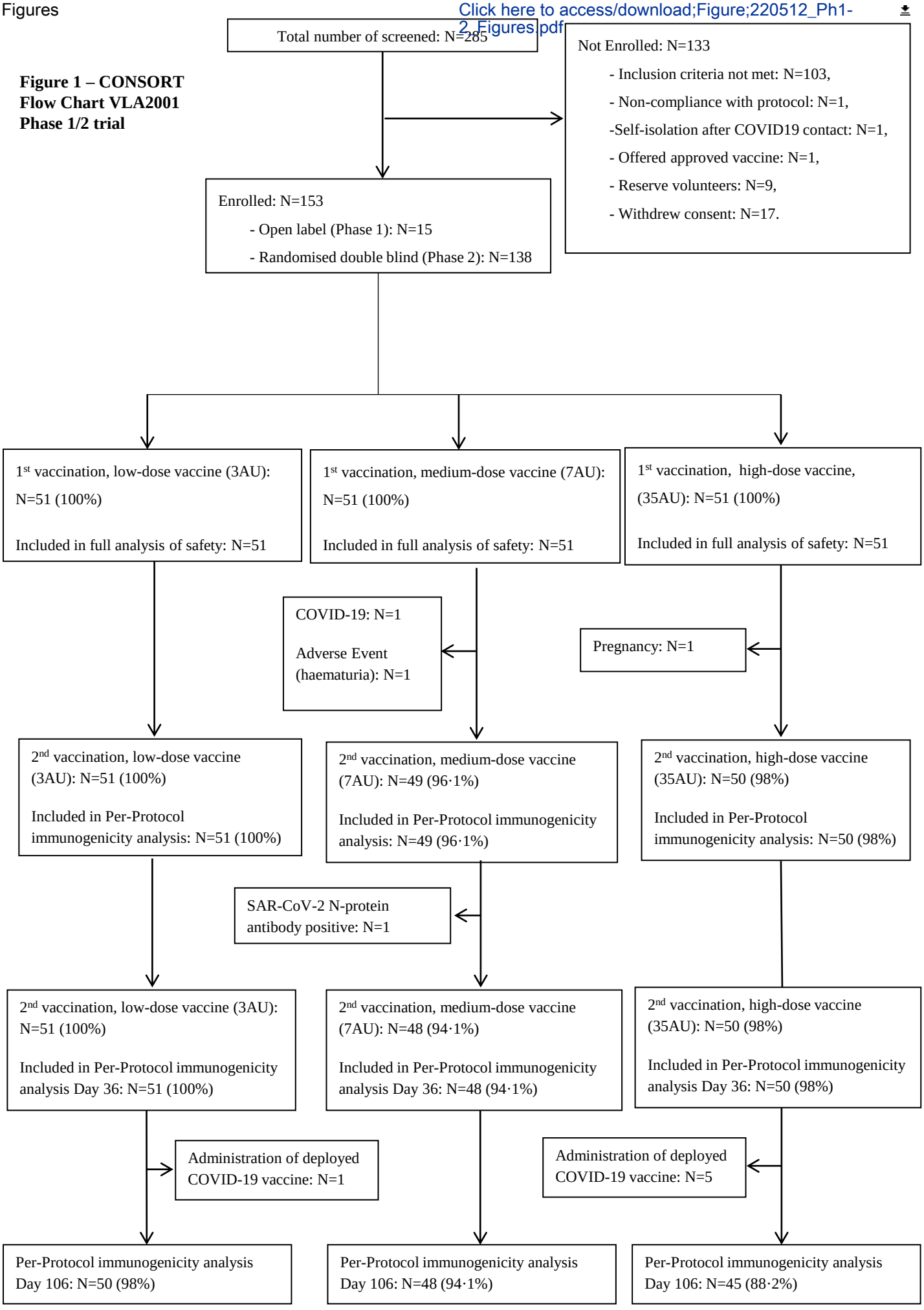
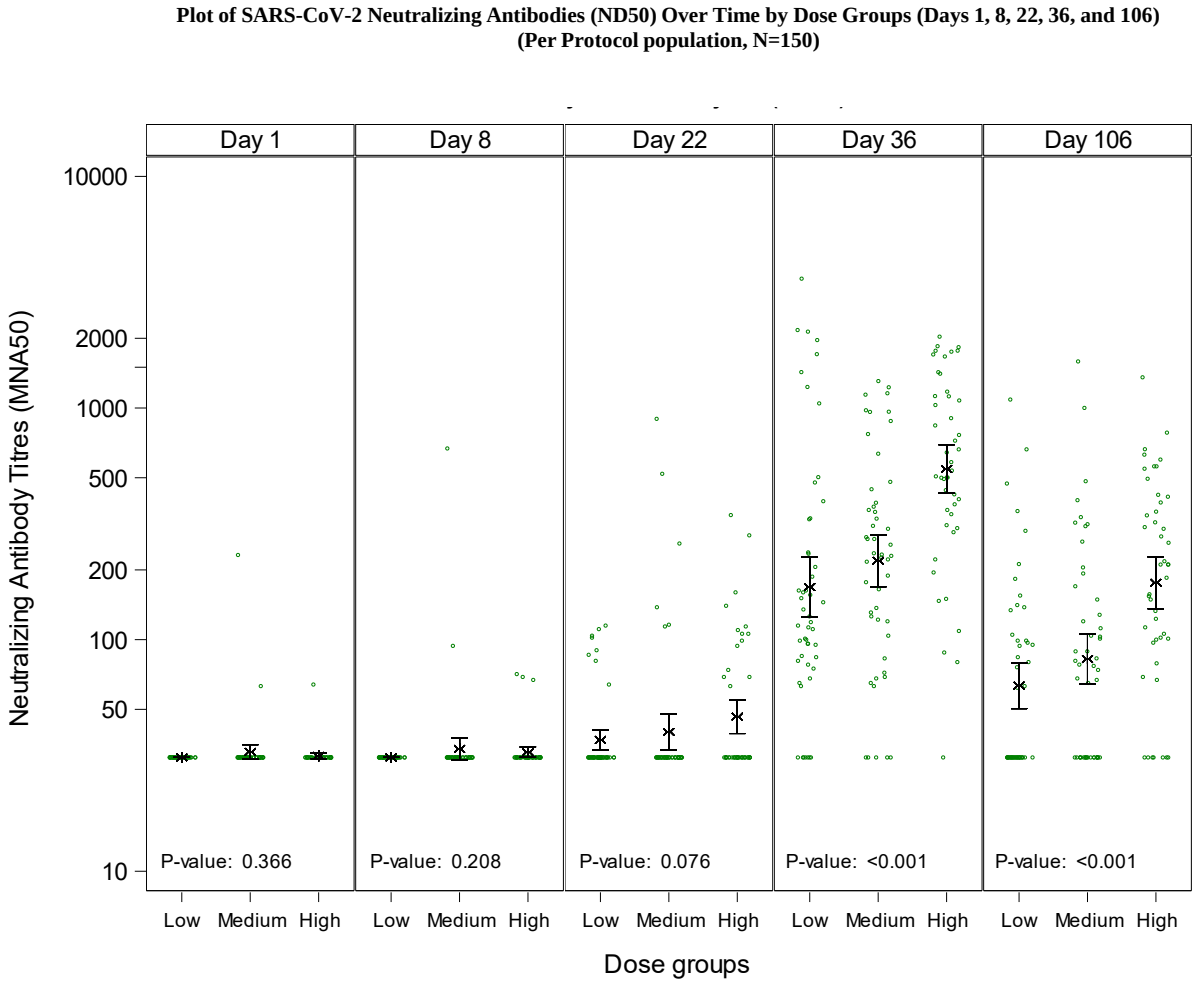


Figure 2: Plot of SARS-CoV-2 Neutralizing Antibodies (ND50) Over Time by Dose Groups (Days 1, 8, 22, 36, and 106).



CI=confidence interval; GMT=geometric mean titre; MNA=microneutralization assay; MNA50=50% of microneutralization dilution; ND50=50% neutralizing dilution

Note: The graph shows GMT and 95% CI. The scatter dots are the actual distribution of neutralizing antibody titres.

Day 1: Low N=50; Medium N=49; High N=45

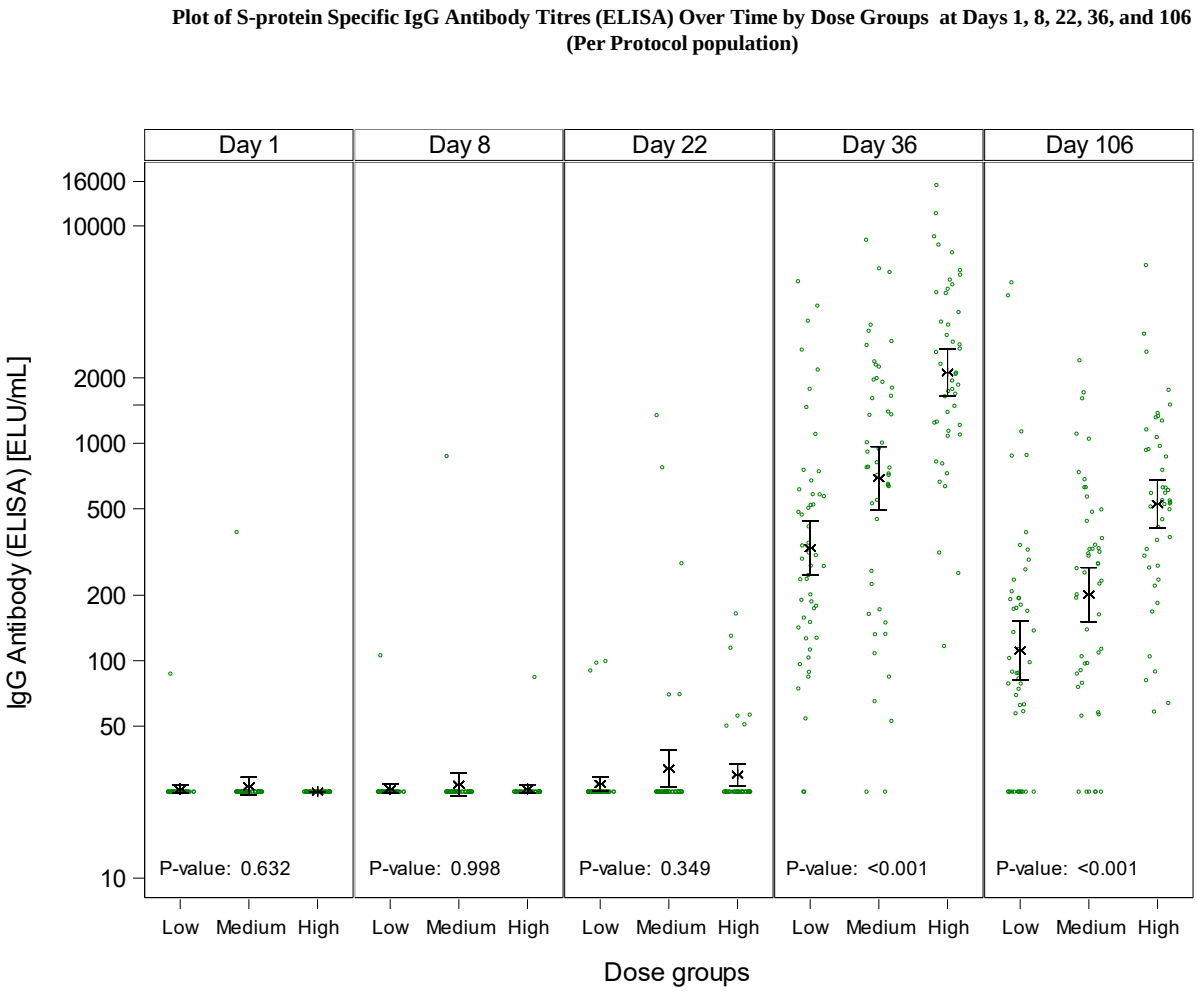
Day 8: Low N=50; Medium N=49; High N=45

Day 22: Low N=50; Medium N=49; High N=45

Day 36: Low N=50; Medium N=49; High N=45

Day 106: Low N=50; Medium N=49; High N=45

Figure 3 : Plot of S-protein Specific IgG Antibody Titres (ELISA) Over Time by Dose Groups at Days 1, 8, 22, 36, and 106 (Per Protocol population)



CI=confidence interval; GMT=geometric mean titre; MNA=microneutralization assay; MNA50=50% of microneutralization dilution; ND50=50% neutralizing dilution

Note: The graph shows GMT and 95% CI. The scatter dots are the actual distribution of neutralizing antibody titres.

Day 1: Low N=50; Medium N=49; High N=45

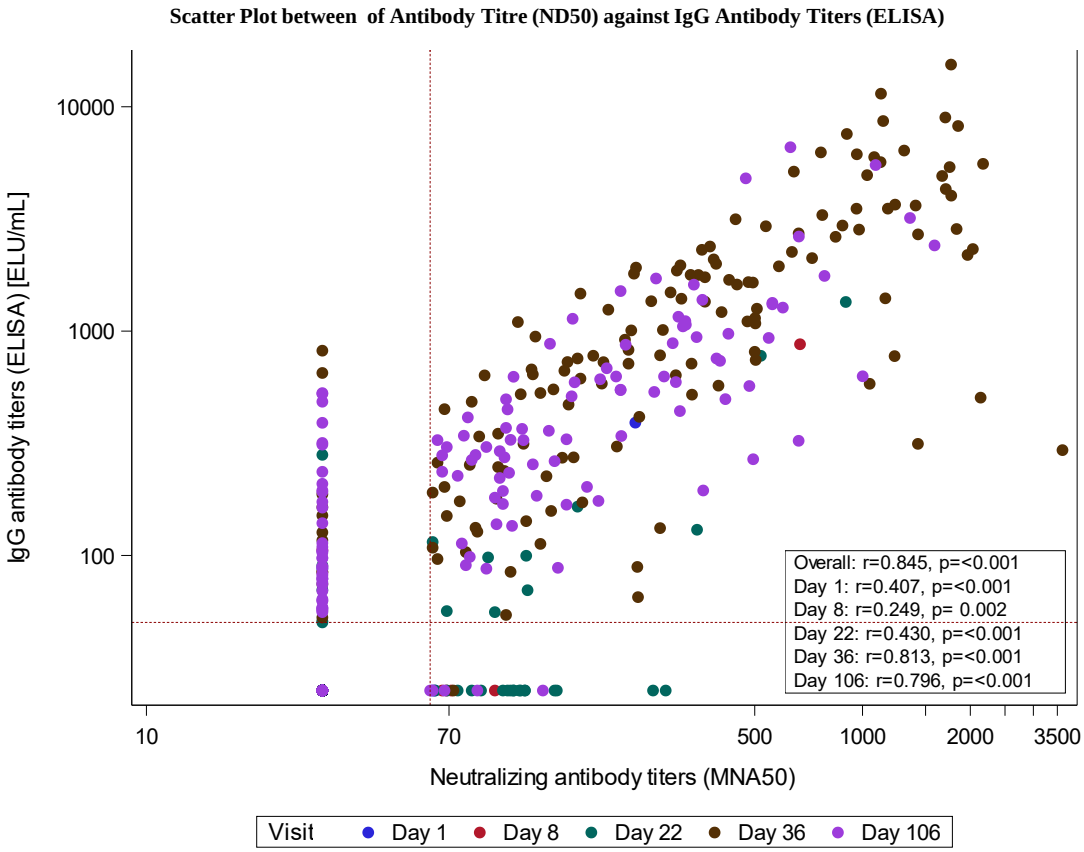
Day 8: Low N=50; Medium N=49; High N=45

Day 22: Low N=50; Medium N=49; High N=45

Day 36: Low N=50; Medium N=49; High N=45

Day 106: Low N=49; Medium N=49; High N=45

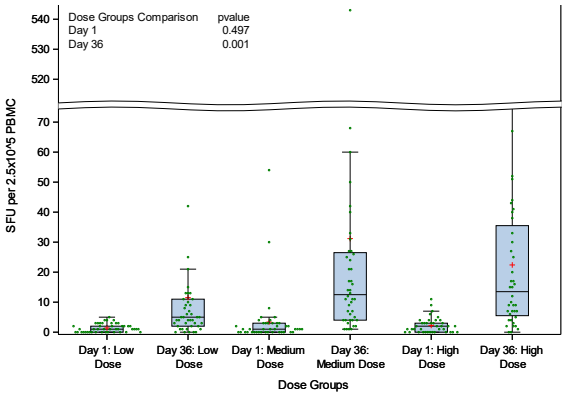
Figure 4 : Correlation between neutralizing and IgG Antibody titers.



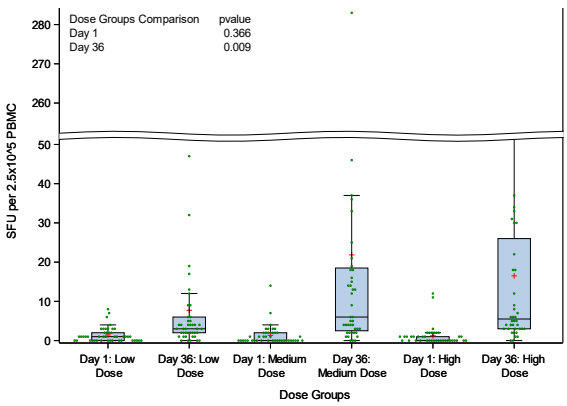
ELISA=enzyme-linked immunosorbent assay; IgG=immunoglobulin gamma; ND50=50% neutralizing dilution
Note: The scatter plot shows the correlation between results of ELISA (ELU/mL) and MNA (ND50). Spearman rank correlation coefficient (r) between ELISA (ELU/mL) and MNA (ND50) and p-value for testing the significance of the correlation coefficient is also presented in the plot. The red dotted lines present the limit of detection for ELISA (50·3 ELU/mL) and MNA (ND50=62)

Figure 5 : Cellular immune response (Interferon Gamma)

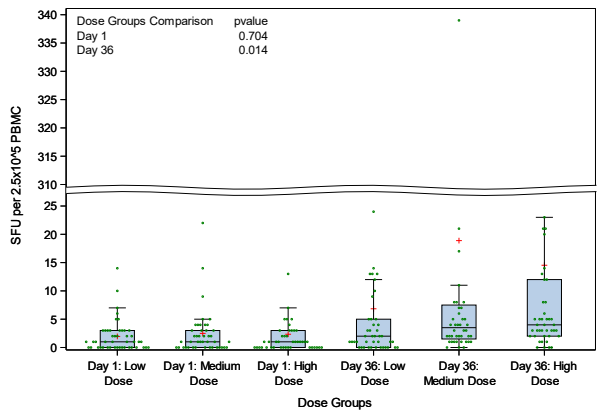
Panel A - Plot of Interferon Gamma Spot Forming Units per $2{\cdot}5 \times 10^5$ PBMC by Dose Groups and Assessment Days for Panel 14 **Spike Protein Full Sequence** (Per-Protocol population)



Panel B - Plot of Interferon Gamma Spot Forming Units per $2{\cdot}5 \times 10^5$ PBMC by Dose Groups and Assessment Days for Panel 3 **Nucleocapsid -protein** (Per-Protocol population)



Panel C - Plot of Interferon Gamma Spot Forming Units per $2{\cdot}5 \times 10^5$ PBMC by Dose Groups and Assessment Days for Panel 4 **Membrane -protein** (Per-Protocol population)



PBMC=peripheral blood mononuclear cells;
SFU=spot forming units
Note: Boxplots show median, lower quartile, and upper quartile; the horizontal line within each box is the median and red plus sign represents the mean value for each group. Green scatter dots are the actual distribution of spot forming units per $2{\cdot}5 \times 10^5$ PBMC within each group.

Note: A sample was considered 'Reactive' at baseline (Day 1) if the normalized SFU ≥ 6 in at least one SARS-CoV-2 specific stimulation panel (i.e. Panel 3, 4, 14) on Day 1.