Immunogenicity and safety of inactivated whole virion Coronavirus vaccine with CpG (VLA2001) in healthy adults aged 18 to 55

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Immunogenicity and safety of inactivated whole virion Coronavirus vaccine with CpG (VLA2001) in healthy adults aged 18 to 55: a randomised phase 1 /2 clinical trial

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Abstract

Background

We assessed the safety, tolerability and immunogenicity of VLA2001 is a whole-virion inactivated

SARS-CoV-2 vaccine adsorbed to alum with a toll-like receptor 9 agonist adjuvant in healthy

volunteers aged 18-55.

Methods

The first 15 participants were enrolled, in groups of 5, to receive two doses, separated by 21 days, of

one of three dose concentrations, administered intramuscularly. 138 further participants were

randomised 1:1:1 to receive the same 3 dose concentrations, in a double blinded manner. Primary

outcomes were solicited adverse reactions 7 days after each vaccination and neutralising antibody

geometric mean titres (GMT) against SARS-CoV-2, 2 weeks after the second vaccination (day 36),

measured by live microneutralisation assay against wild-type virus (MNA50). Secondary outcomes

included unsolicited adverse events, and humoral and cellular responses at day 36, measured by IgG

ELISA against Spike protein and interferon-γ secreting T-cells by ELISpot stimulated with multiple SARS-CoV-2 antigens. (ClinicalTrials.gov NCT04671017, ISRCTN 82411169)

Findings

Between December 16, 2020 and January 21, 2021, 153 participants were enrolled and randomised evenly between the dose groups. The rates of solicited reactions were similar after the first and second doses and between the three dose groups. The most frequent local reactions were tenderness (58·2%) and pain (41·8%) and systemic reactions were headache (46%) and fatigue (39·2%).

In the high dose group, two weeks following the second dose, the geometric mean titres were 530.4 (95% CI: 421·49, 667·52) for neutralizing antibodies and 2147·9 (95% CI: 1705·98, 2704·22) for S-binding antibodies. There was a dose dependent response with 90·0% (95% CI:78·0%.,97·0%) seroconversion (4-fold rise) at day 36 in the high dose group, which was significantly higher than rates in both the medium (73.5%; 95% CI: 59%,85%), CIs) and low dose (51%; 95%CI: 37%,65%) rate, CIs) groups (both p < 0.001). Antigen-specific interferon-γ T-cells reactive against the S, M and N proteins were observed in 76, 36 and 49% of high dose recipients, respectively.

Interpretation

 VLA2001-201 was well tolerated and produced both humoral and cellular immune responses, with a clear dose-response effect.

Funding

Introduction

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to cause considerable mortality and morbidity worldwide.[1] Several vaccines against SARS-CoV-2 have been given emergency licenses around the world and millions of individuals have been vaccinated.[2] Most of these licensed vaccines induce immunity to the SARS-CoV-2 Spike protein which is the viral protein primarily involved in host receptor recognition, attachment and entry into cells.[2]

Inactivated vaccines have been used extensively over past decades to provide protection against a number of pathogens. There are 16 inactivated COVID-19 candidate vaccines in clinical evaluation with some already deployed in many countries and administered to millions of people.[3] Several studies demonstrate that inactivated vaccines containing aluminium adjuvant induce neutralising antibody responses and have good safety profiles.[4-7]. A desirable characteristic for any COVID-19 vaccine candidate is the ability to induce T-cell responses. Most whole-virion inactivated vaccines are adjuvanted with alum alone, and induce only limited cell-mediated responses.

VLA2001 is a highly purified, inactivated, whole virion, SARS-CoV-2 vaccine, formulated with aluminium hydroxide combined with cytosine phospho-guanine (CpG 1018; Dynavax Technologies Corporation). The immunogenicity of VLA2001 has been demonstrated in murine studies (unpublished data) and was enhanced by the addition of CpG 1018, a toll-like receptor 9 (TLR-9) agonist, stimulating production of innate pro-inflammatory cytokines and a pronounced Th1

immune response. This adjuvant has an established safety profile and is contained in a licensed hepatitis B vaccine.[8]

The aim of the study was to assess safety, reactogenicity and immunogenicity of this novel coronavirus vaccine and to establish the optimal dose to take forward into further clinical development.

Methods

Study design and participants

This report describes results from the first 36 days of follow up, including two weeks after a second dose of vaccine, in an ongoing safety and dose-finding immunogenicity trial. Following an initial open-label dose escalation phase, the second phase of the study had a randomised, double-blind parallel group design with a 1:1:1 allocation to three dose levels. Healthy adult volunteers aged 18-55 years were recruited either through local advertisement or a national vaccine volunteer registry. All participants were screened with full medical history and physical examination and blood and urine samples were sent to assess HIV, hepatitis B and C, SARS-CoV2 antibodies, full blood count, clotting function, inflammatory markers, liver and renal function and urinary abnormalities (including blood, protein and glucose). All women of childbearing potential had a serum HCG test performed at screening and urine pregnancy tests at all subsequent visits. Subjects with acute febrile or chronic disease, laboratory-confirmed SARS-CoV2 infection or who participated in other SARS-CoV-2 vaccine clinical studies were excluded; for a full list of exclusion criteria refer to supplementary material.

Written informed consent was obtained 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.

Randomisation and Blinding

The phase 2 study participants were randomly assigned (1:1:1) to receive either the 3AU (low), 7AU (medium) or 35AU (high dose) of VLA2001. The randomisation code was generated by the study statistician and allocation was performed within a secure web platform. The investigational medicinal product (IMP) was provided to study sites in packaging that was identical for all strengths of the vaccine. IMP allocation was based on an identifier linked to the randomisation, therefore as there was no visual difference between the doses, study staff and participants remained blinded to dose allocation.

Procedures

VLA2001 was produced using a highly purified, whole virus, SARS-CoV-2 strain derived from a Chinese tourist from Hubei, diagnosed with COVID-19 in a hospital in Rome. The virus was cultured in Vero cells and inactivated with β-propiolactone. VLA2001 is adjuvanted with cytosine phospho-guanine (CpG) 1018, a licensed adjuvant, in combination with aluminium hydroxide. The vaccine was manufactured at Valneva, Scotland, according to current Good Manufacturing Practice as described in the Investigational Medicinal Product Dossier and approved by the regulatory agency in the UK. The antigen contents of the vaccines were evaluated using Spike protein ELISA and has been confirmed in the final product to be 3AU, 7AU and 35AU for the low, medium and high doses respectively. The vaccine was given as a 2-dose schedule separated by 21 days. The active substance

was combined with CpG 1018 1mg/dose to reach final concentrations in 0.5ml immediately before administration. Vaccines were administered by intramuscular injection into the deltoid.

The initial participants were recruited into an open label, dose escalation study, in December 2020, to receive either the low (n=5), medium (n=5) or high dose (n=5) vaccine. The first participant in each dose group was immunised and then observed for 3 hours and interviewed after 24 hours by telephone, to assess for adverse reactions, before the remaining participants in each dose group were vaccinated sequentially, with each one observed for one hour before the next was vaccinated. These participants were then revaccinated 3 weeks later following the same procedures.

A double-blinded randomised stage was initiated after the safety data from the initial stage of the study, up to 3 days after the last high dose vaccination, had been reviewed by an independent data safety monitoring committee to ensure none of the pre-specified stopping rules had been met.

Participants received a vaccine on Day 0 and Day 21 and were asked to record solicited and unsolicited adverse events using electronic diaries during the 7 days after vaccination. They were provided with a digital thermometer to measure oral temperature every evening from the day of vaccination for 7 days. Solicited local events at the site of vaccination were pain, tenderness, erythema and induration/swelling while systemic events were nausea/vomiting, headache, fatigue and myalgia. Unsolicited events, adverse events of special interest (AESI) and serious adverse events (SAE) were collected up to day 36. AESI were defined as COVID-19 manifestations and complications associated with COVID-19, due to the theoretical risk for disease enhancement, as well as immune-mediated disorders due to the addition of the CpG 1018 adjuvant. Changes in laboratory parameters were assessed. All adverse events and abnormal laboratory parameters were graded in accordance with the FDA's toxicity scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials.[9] T-cell responses were classified as reactive if 6 or more spot forming units per 2×10^5 peripheral blood mononuclear cells (PBMC) were present once counts in control cells had been subtracted.

Blood samples were taken on days 0, 8, 21 and 36. Antibody responses at these time points were measured using an immunoassay for IgG to full length S-protein (Nexelis, Canada) and a live microneutralisation assay MNA_{50} against the Victoria strain performed by Public Health England (Porton Down, UK).[10] Cellular immunity against S-protein, Nucleocapsid protein and Membrane protein was assessed at Oxford Immunotec using T-Spot Discovery SARS-CoV-2 (Oxford, UK).[11]

Outcomes

The primary outcome was the frequency and severity of solicited adverse events (AE) within 7 days of first or second vaccination. Secondary outcomes in this report were the number and percentage of participants with unsolicited AE related AE, AESI and SAE by severity up to day 36.

The primary immunogenicity outcome was the geometric mean titres (GMT) for SARS-CoV-2 neutralising antibodies at day 36. Secondary immunogenicity outcomes included GMT of Spike protein binding and neutralising antibodies at all time points. T-cell responses (spot forming units per 2×10^5 PBMC) were included as exploratory outcomes. The full list of outcomes is available in the protocol (supplementary material).

Statistical analysis

This is a descriptive study and formal power calculations were not performed. A total of 150
participants was agreed with regulators to be sufficient for initial safety data. The safety analysis
included all participants who received at least a single dose of vaccine, the full analysis set. The
immunogenicity analysis was performed on a per-protocol analysis set (PPAS), which excluded
participants who received less than two vaccinations, received the wrong study medication or who
fulfilled the exclusion criteria. The results for all participants were combined for analysis and
reporting including the participants who were enrolled in an open label, non-randomised 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 Fisher-Freeman-Halton exact test and p-values were presented
for this test.   GMTs (CI) were be calculated by taking the antilogarithm of the mean (CI) of the log10
transformed titre. Also, p-values from Kruskal Wallis test were calculated to check if the results were
significantly different among dose groups at 5% level of significance. If the test suggested
significance, then a pairwise group comparison was perfomed using Dwass, Steel, Critchlow-Fligner
(DSCF) multiple comparisons post-hoc procedure to determine which pair of dose groups differed
significantly. Secondary immunogenicity analyses include comparison of the SCRs on days 21 and 36
using Fisher-Freeman-Halton exact test. If overall group difference were 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 difference were calculated with Fisher's exact test.

Seroconversion was defined as at least a four-fold increase in GMT from baseline. Statistical

analysis using SAS® version 9.4 was performed by Valneva and were independently verified by LD

using R version 4.0.2.

An independent data and safety monitoring board provided safety oversight. This trial is registered

with ClinicalTrials.gov, NCT04671017 and with ISRCTN, 82411169

Role of the funding source

The funder had no role in the design, delivery or analysis of the study

Results

Between 16th December 2020 and 21st January 2021 285 individuals aged between 18 and 55 were

screened and 153 were enrolled in the study, with 51 participants in each dose group. All

randomised participants received the first dose of vaccine but 3 participants did not receive a second

dose due to pregnancy, diagnosis of COVID-19 and investigator decision not to administer the

second dose following an adverse event (haematuria) considered to be treatment-unrelated, figure

1. The mean age of participants was 33·5 years. 46% were female and 93·5% were white, table 1.

There were no significant differences in reported reactions between the first and second vaccine

doses or the different dose strengths. Overall, 66·7% of vaccinees reported at least one solicited

injection site reaction (68·6% low, 60·8% medium and 70·6% high dose groups) with tenderness and

injection site pain most commonly reported, table 2a. Overall, 69·3% of vaccinees reported at least

one solicited systemic reaction (72·5% low, 62·7% medium and 72·5% high dose groups) with

headache, fatigue and muscle pain most frequently reported, table 2a. Only two participants, both

in the low dose group, reported a fever post-vaccination. Three reactions reported by 2 participants

were classified as severe, one participant with headache and fatigue and another reported fatigue,

both participants recovered within

24 hours. All other local or systemic reactions were reported as mild or moderate (table 2b) and the

majority resolved within 7 days of vaccination. Unsolicited adverse events, including laboratory

abnormalities thought to be related to vaccination were reported in 27 (17·6%) participants, with

23·5%, 13·7% and 15·7% reported in the low, medium and high dose groups respectively. The most

common laboratory abnormality was a rise in the red blood cell/erythrocyte sedimentation rate

(ESR), which was reported in 11 (7·2%) of participants at a single centre. In addition, neutropenia

was observed in 2 participants (1·3%) and eosinophilia (0·7%) and thrombocytopenia (0·7%) in one

participant each up to Day 36. There were two cases of COVID-19, confirmed by nucleic acid

amplification testing. One mild COVID-19 case occurred in a participant in the medium dose group,

16 days after the first vaccination. The other COVID-19 case was moderate in severity in a participant

in the low dose group 4 days after the second vaccination. 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

administered without further event. As per protocol definition, any AESI was treated as serious

adverse event in this study. However, no other AESI nor SAE was reported up to day 36.

In the SARS-CoV-2 neutralising MNA_{50} assay, the highest median GMT recorded to date were

reached on day 36 in all dose groups, in a dose dependent manner (high dose group, 520 [95% CI

421·49, 667·52]; medium dose, 222·3 [95% CI 171·84, 287·67]; low dose, 161·1 [CI 121·35, 213·82])

shown in table 3. The MNA_{50} GMT were significantly higher in the high dose group compared to both

the low and medium dose groups (p < 0·001). The seroconversion rates of neutralising antibodies by

day 36 were 90·0% (95% CI:78·0%.,97·0%) in the high dose group, 73.5% (95% CI: 59%,85%), in the

medium dose group and 51% (95%CI: 37%,65%) in the low dose group.

A similar dose dependent response, with a peak response at day 36, was observed in anti-spike-IgG

GMT measured by ELISA (high dose, 2147·9 [95% CI 1705·98, 2704·22]; medium dose, 691·6; [95% CI

494·.91, 966·.52]; low dose, 325; [95% CI 245·45, 430·36]), table 4. The anti-spike IgG GMT were

significantly higher in the high dose groups in comparison to the other dose groups (p < 0.001). The

neutralisation titres at day 36 correlated with anti-S-protein specific binding IgG (r=0·79 and p <

0·001), shown in figure 2. Median IFN-γ secreting ELISpot responses to Spike (S), Nucleocapsid (N)

and Membrane (M) protein increased from baseline in all dose groups by day 36. In the high dose

group, a reactive response was observed in 75% (34/45 participants) against S-protein,(figure 3),

36% (16/45)against M-protein and 49% (22/45) against N-protein,(supplementary material).

Discussion

The vaccine candidate VLA2001 evaluated in this phase 1/2 study is a second wave vaccine candidate

which includes the additional immune modulator CpG1018 to further broaden immune stimulation.

The results from this study indicate that this vaccine candidate was able to induce both robust

humoral and cellular immune responses. In general, inactivated vaccines adjuvanted with aluminium

salts only induce minimal cellular immune responses and available data on SARS-CoV-2 vaccines of

this kind demonstrate only a limited T-cell response.[4-7] Zhang et al, reported poor T-cell responses to

viral S-protein.[15] In contrast, in a phase 1 study of BB152, a whole virion, inactivated vaccine

formulated with a toll like receptor 7/8 agonist, CD4+ and CD8+ T-cell responses were detected in a subset of 16 participants.[7]

CpG 1018 is licensed as a vaccine adjuvant for a hepatitis B vaccine. The pre-clinical and clinical studies for that vaccine demonstrated that the addition of CpG 1018 increased antibody concentrations and resulted in robust T cell responses.[8] Similarly, the results presented in this study show that the vaccine candidate VLA2001 was able to induce antigen specific T-cell responses against the S-protein, M and N protein, in many of the participants who received the high dose vaccine. Induction of both humoral and cellular immune responses against viral antigens beyond the S-protein may be beneficial for cross-protection against variants by broadening epitope coverage and immune effector mechanisms. [12,13]

There was a clear dose dependent increase in both neutralising and spike protein binding antibodies with a high correlation between them. Ascending concentrations were recorded 2 weeks after the second dose of vaccine, with seroconversion for S-protein binding antibodies of up to 100%. Three weeks after a single dose only 6 (9%) of all participants in all dose groups showed seroconversion. Antibody concentrations after a single dose may have reached higher concentrations if measured at a longer interval after vaccination as vaccine-induced antibody concentrations have been reported to peak about 4 weeks after vaccination for other COVID-19 vaccines after each dose. [4, 14]

After the initial pandemic response, reactogenicity and tolerability may become a more important feature of booster vaccines, particularly if they are to be used annually and a choice of vaccines is available, to help maintain uptake and compliance. VLA2001, given as a two-dose schedule with a 3-week interval was safe and well tolerated at all dose levels and no safety concerns were identified by an independent Data Safety Monitoring Board. Importantly, only two participants reported severe solicited AEs and there were no severe vaccine-related unsolicited events as well as no serious treatment-related adverse events. Of note, no cases of fever were reported in the two highest dose groups. Currently approved viral vector and mRNA vaccines have been reported to cause fever and

chills in between 16% and 24% of individuals depending on the age and dose (first or second). [15,16]
Furthermore, using the same approach as used for the proven and well-established annual
modification of inactivated influenza vaccines, an inactivated vaccine could be particularly well
suited for use as an annual booster vaccine.

Our study has several limitations. Firstly, the population studied was young, healthy adults, who
typically have higher immune responses post vaccination than older adults. While this is a classical
approach in Phase 1/2, it will be necessary and important to include an older population in future
trials. Secondly, we did not study the response to immunization in subjects seropositive for SARS-
CoV-2, so we cannot yet quantify any safety issues or a potential booster effect of the first dose that
might be present in such individuals, as has been seen for other vaccines Thirdly, we have only
assessed antibody titres at 2 weeks after the second dose at the moment. Further follow-up of
participants is ongoing to provide immunogenicity data from time points later after the primary
series.

The highest dose group has been selected for further clinical development based on comparable
safety and superior immunogenicity results. At the time of this publication, the sponsor has initiated
a comparative, immunogenicity Phase 3 trial that will aim to predict vaccine efficacy by employing a
primary endpoint of superiority of the GMT ratio of neutralizing antibody titres following 2 doses of
VLA2001 compared to 2 doses of AZD1222.[17]

Contributors

AF is the chief investigator. RL, CD, SF and CG were the study site principal investigators. RL and CT

prepared this report. All authors critically reviewed and approved the final version.

<u>Data sharing</u>

The study protocol is provided in the appendix.

Declaration of interests

CT, BQ, KD, SE-L and RH are all employees of Valneva. RL has worked on trials funded by Aztra

Zeneca and Janssen but receives no personal financial payment for this work. AF is a member of the

Joint Committee on Vaccination and Immunisation and Chair of the WHO European Technical

Advisory Group of Experts (ETAGE) on Immunisation. He is an investigator and/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 GSK, VPI, Takeda and Bionet Asia. He receives no personal remuneration or benefits for

any of this work. 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

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GlaxoSmithKline, Novavax, Seqirus, Sanofi, Medimmune, Merck and Valneva vaccines and

antimicrobials. He receives no personal financial payment for this work.

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Research in context

Evidence before this study

We searched Pubmed for research articles published from database inception until April 13th 2021,

with no language restrictions using the terms "inactivated", "SARS-CoV-2", "vaccine", AND "clinical

trial". There are studies in preprint which have not been included in this evidence review. We

identified clinical trial data on six other inactivated SARS-CoV-2 vaccines. A dose-escalation study of

a whole virion inactivated vaccine made using a SARS-CoV-" (CN02) strain vaccine, adsorbed onto

aluminium hydroxide (Coronavac, Sinovac Life Sciences, Beijing), was tested in health adults aged 18

to 59 in a two-dose regimen. seroconversion rates of neutralising antibody were reported as 92%

and 98%, the 3µg and 6µg groups, respectively. T-cell responses, as measured by T-cell ELISpot were

low. The overall incidence of adverse reactions was 29%. BBIBP-CorV (Sinopharm, Beijing), a whole

virion inactivated vaccine with aluminium hydroxide adjuvant was tested with 3 different

concentrations and as a single or two dose regimen with varying dose intervals. The intermediate

dose (4 µg) given on days 0 and 21 or 0 and 28 was found to produce higher neutralisation titres

than a single 8 µg dose or a dose administered on days 0 and 14. Up to 46% of participants

experienced an adverse reaction in the first 7 days after vaccination. No T-cell responses formally

reported but authors report that preliminary results suggest minimal evidence of T-cell activation.

An interim analysis of an inactivated vaccine with aluminium of 2 randomised control trials that

included 320 participants who received one of the three doses as either a 3-dose schedule (days 1,

28 and 56) or a two-dose schedule (days 0 and 14 or days 0 and 21). The vaccine was shown to

produce higher neutralising antibodies when given at 21 or 28 days compared to 14 days and a third

dose resulted in further increase in neutralising antibody titres. The incidence of adverse reactions

was reported as 15%. No discernible T-cell response was noted through the measurement of T-cell

subsets or the evaluation of cytokine profiles. A phase 1, investigating a vaccine alum adsorbed,

inactivated using a patented two step deactivation process was evaluated as a 2-dose regimen given

at an interval of either 14 or 28 days at three different dose ranges (Institute of Medical Biology and

Chinese Academy of Medical Sciences). Adverse reactions were reported in 4.2% to 33.3% of

participants depending on dose group and interval. Seroconversion defined as a fourfold increase in

neutralising antibody concentration after vaccination was between 54.2% to 100%. A phase 1 / 2

trial of an inactivated, alum adsorbed KCONVAC vaccine was initiated with dose escalation from 5 to

10ug in 60 healthy participants with both the 5µg and 10µg doses used in the phase 2. In both

phases the vaccines were administered as a two-dose regimen administered either 14 days or 28

days apart. Seroconversion was reported in 83% to 100% in the 5µg and 10µg dose group

respectively, across both phases of the study. Reactive T-cell IFN- γ-ELISpot were reported in 57% to

63% in the 5µg and 10µg dose group, respectively, 14 days after the first dose of vaccine. Finally,

BBV152, an alum- adjuvanted inactivated vaccine combined with a TL7/8 agonist, given as a 2-dose

regiment, 2 weeks apart and two different doses with and without the TL7/8 agonist and an

aluminium alone group. The rates of seroconversion ranged between 82.8% and 87.9% with no

significant difference in the groups who received the TL7/8 agonist containing vaccine. In contrast, in

small subsets (13 participants) in each vaccine group cellular responses were observed in the group

receiving the TLR 7/8 containing vaccine but not in the vaccines without this. There were no safety

concerns reported in any of these trials. Peer reviewed data on efficacy is limited however a recent

manuscript of a randomised clinical trial including two alum-adjuvanted, inactivated vaccines, which

demonstrated an efficacy between 72.8% and 78.1% against laboratory-confirmed symptomatic

COVID-19 disease.   A prospective, observation study has also recently demonstrated the

effectiveness of CoronaVAC as 65.9% against laboratory confirmed disease and 87.5% against hospitalisation.

Added value of this study

This will be the seventh published study of a vaccine against SARS-CoV-2 which has been tested in healthy adults aged 18 to 55 and the first using the TLR9 agonist, CpG. The vaccine was shown to be safe and well tolerated. Neutralising and binding antibodies against SARS-CoV-2 were induced at all doses evaluated after 2 doses of vaccine and increased in a dose dependent manner. Cellular immune responses were also demonstrated by 14 days after the second dose of vaccine. The cellular response is believed to be stimulated by the addition of the CpG adjuvant, a Toll like receptor 9 agonist.

Implications of all the available evidence

Inactivated vaccines against SARS-CoV-2 contribute to the portfolio of COVID-19 vaccines that are required to ensure a resilient global vaccine supply. An important limitation of inactivated vaccines is can be weak cellular immunity however the addition of suitable adjuvants such as CpG may overcome this limitation. Further assessment of the efficacy and immunogenicity of this vaccine in relation to an approved comparator vaccine is under way.

	Low Dose (N=51) n (%)	Medium Dose (N=51) n (%)	High Dose (N=51) n (%)	Overall (N=153) n (%)
Age at the time of informed consent (years)				
n	51	51	51	153
Median	31	33	29	32
Min, Max	21, 53	21, 55	18, 54	18, 55
Sex				
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)
Ethnicity				
White	46 (90·2)	51 (100·0)	47 (92·2)	144 (94·1)
Asian	1 (2·0)	0	0	1 (0·7)
Mixed	0	0	4 (7·8)	4 (2·6)
Latino*	4 (7·8)	0	0	4 (2·6)

*Latino includes individuals who classified themselves as Latin, Latin American and Latino

Table 1 Baseline demographics and characteristics

Adverse Event/Severity	Low Dose (N=51) n (%)	Medium Dose (N=51) n (%)	High Dose (N=51) n (%)	Overall (N=153) n (%)
Participants with at least one Solicited Injection Site Reaction	35 (68·6)	31 (60·8)	36 (70·6)	102 (66·7)
Mild (Grade 1)	32 (62·7)	27 (52·9)	32 (62·7)	91 (59·5)
Moderate (Grade 2)	3 (5·9)	4 (7·8)	4 (7·8)	11 (7·2)
Injection Site Tenderness	32 (62·7)	23 (45·1)	34 (66·7)	89 (58·2)
Mild (Grade 1)	30 (58·8)	20 (39·2)	33 (64·7)	83 (54·2)
Moderate (Grade 2)	2 (3·9)	3 (5·9)	1 (2·0)	6 (3·9)
Injection Site Pain	19 (37·3)	21 (41·2)	24 (47.1)	64 (41·8)
Mild (Grade 1)	17 (33·3)	20 (39·2)	21 (41·2)	58 (37·9)
Moderate (Grade 2)	2 (3·9)	1 (2·0)	3 (5·9)	6 (3·9)
Injection Site Itching	3 (5·9)	3 (5·9)	2 (3·9)	8 (5·2)
Mild (Grade 1)	3 (5·9)	3 (5·9)	2 (3·9)	8 (5·2)
Injection Site Swelling	1 (2·0)	0	1 (2·0)	2 (1·3)
Mild (Grade 1)	1 (2·0)	0	0	1 (0·7)
Moderate (Grade 2)	0	0	1 (2·0)	1 (0·7)

Table 2a Injection site reactions 7 days after any vaccine by frequency and severity

Adverse Event/Severity	Low Dose (N=51) n (%)	Medium Dose (N=51) n (%)	High Dose (N=51) n (%)	Overall (N=153) n (%)
Participants with at least one Solicited Systemic Reaction	37 (72·5)	32 (62·7)	37 (72·5)	106 (69·3)
Mild (Grade 1)	26 (51·0)	23 (45·1)	26 (51·0)	75 (49·0)
Moderate (Grade 2)	11 (21·6)	9 (17·6)	9 (17·6)	29 (19·0)
Severe (Grade 3)	0	0	2 (3·9)	2 (1·3)
Headache	28 (54·9)	17 (33·3)	26 (51·0)	71 (46·4)
Mild (Grade 1)	22 (43·1)	14 (27·5)	20 (39·2)	56 (36·6)
Moderate (Grade 2)	6 (11·8)	3 (5·9)	5 (9·8)	14 (9·2)
Severe (Grade 3)	0	0	1 (2·0)	1 (0·7)
Fatigue	23 (45·1)	15 (29·4)	22 (43·1)	60 (39·2)
Mild (Grade 1)	16 (31·4)	10 (19·6)	15 (29·4)	41 (26·8)
Moderate (Grade 2)	7 (13·7)	5 (9·8)	5 (9·8)	17 (11·1)
Severe (Grade 3)	0	0	2 (3·9)	2 (1·3)
Muscle Pain	18 (35·3)	15 (29·4)	17 (33·3)	50 (32·7)
Mild (Grade 1)	15 (29·4)	13 (25·5)	16 (31·4)	44 (28·8)
Moderate (Grade 2)	3 (5·9)	2 (3·9)	1 (2·0)	6 (3·9)
Nausea/Vomiting	5 (9·8)	7 (13·7)	6 (11·8)	18 (11·8)
Mild (Grade 1)	5 (9·8)	6 (11·8)	5 (9·8)	16 (10·5)
Moderate (Grade 2)	0	1 (2·0)	1 (2·0)	2 (1·3)
Fever/Body Temperature (T > 38°C)	2 (3·9)	0	0	2 (1·3)
Mild (Grade 1)	0	0	0	0
Moderate (Grade 2)	2 (3·9)	0	0	2 (1·3)

Table 2b Solicited adverse reactions 7 days after any vaccine by frequency and severity

Visit		Low Dose (N=51)	Medium Dose (N=49)	High Dose (N=50)	Overall (N=150)

		n (%)	n (%)	n (%)	n (%)
Day 1		51	49	50	150
	GMT (95% CI)	29·0 (29.00, 29.00)	30·7 (28.51, 33.15)	30·0 (28.83, 31.23)	29·9 (29.08, 30.73)
	Min, Max	29·0, 29·0	29·0, 232·0	29·0, 72·0	29·0, 232·0
	p-value (Overall Dose Groups Comparison)				0.349
Day 22		50	48	48	146
	GMT (95% CI)	35·9 (32·31, 39·93)	38·3 (31·92, 46·06)	46·5 (38·79, 55·66)	39·9 (36·45, 43·76)
	Min, Max	29·0, 115·0	29·0, 640·0	29.0, 359·0	29·0, 640·0
	p-value (Overall Dose Groups Comparison)				0.089
Day 36		51	48	50	149
	GMT (95% CI)	161·1 (121·35, 213·82)	222·3 (171·84, 287·67)	530·4 (421·49, 667·52)	266·6 (226·79, 313·34)
	Min, Max	29·0, 2171·0	29·0, 1307·0	29·0, 2033·0	29·0, 2171·0
	p-value (Overall Dose Groups Comparison)				<0·001
	p-value (Low Dose vs Medium dose) [2]				0·204
	p-value (Medium Dose vs High Dose) [2]				<0·001
	p-value (Low Dose vs High Dose)				<0·001

GMT: Geometric Mean Titre, CI: Confidence Interval; Values below Limit of Quantification (58) are set to 29.0

Table 3 SARS-CoV-2 Neutralising Antibody Titres (ND40) at baseline prior to vaccination, 22 days after 1st vaccination and 14 days after second vaccination;

Visit		Low Dose (N=51) n (%)	Medium Dose (N=49) n (%)	High Dose (N=50) n (%)	Overall (N=150) n (%)
Day 1		51	49	50	150
	GMT (95% CI)	25·6 (24·59, 26·69)	26·4 (24·07, 29·05)	25·0 (25·00, 25·00)	25.7 (24·84, 26·54)
	Min, Max	25·0, 87·2	25·0, 390·8	25·0, 25·0	25·0, 390·8
	p-value (Overall Dose Groups Comparison)				0.604
Day 22		51	49	50	150
	GMT (95% CI)	27·1 (25·10, 29·16)	31·9 (26·15, 38·86)	30·0 (26·92, 33·48)	29·6 (27·35, 31·93)
	Min, Max	25·0, 99·7	25·0, 1348·6	25·0, 165·0	25·0, 1348·6
	p-value (Overall Dose Groups Comparison)				0·293
Day 36		51	49	50	150
	GMT (95% CI)	325·1 (245·45, 430·46)	691·6 (494·91, 966·52)	2147·9 (1705·98, 2704·22)	780·6 (643·85, 946·37)
	Min, Max	25·0, 5566·1	25·0, 8637·6	116·8, 15419·5	25·0, 15419·5
	p-value (Overall Dose Groups Comparison)				<0·001
	p-value (Low Dose vs Medium dose)				0·004
	p-value (Medium Dose vs High Dose)				<0·001
	p-value (Low Dose vs High Dose)				<0·001

GMT: Geometric Mean Titre, CI: Confidence Interval, Values below Limit of Quantification (50.3) are set to 25.0

Table 4 IgG antibody Titres Against SARS-COV-2 S-protein at baseline prior to vaccination, 22 days

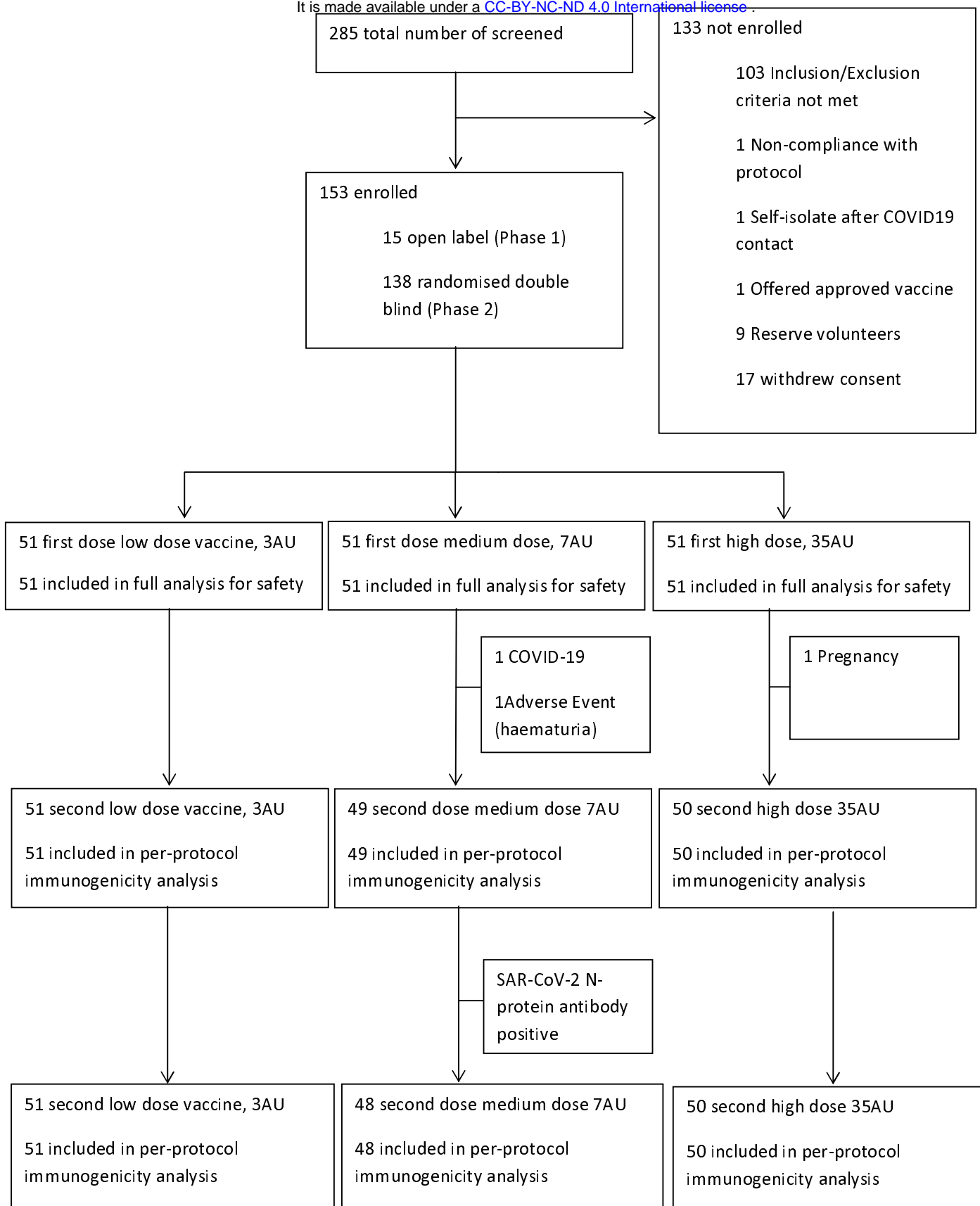after 1st vaccination and 14 days after second vaccination (day 36)

285 total number of screened

153 enrolled 15 open label (Phase 1) 138 randomised double blind (Phase 2)

133 not enrolled 103 Inclusion/Exclusion criteria not met 1 Non-compliance with protocol 1 Self-isolate after COVID19 contact 1 Offered approved vaccine 9 Reserve volunteers 17 withdrew consent

51 first dose low dose vaccine, 3AU 51 included in full analysis for safety	51 first dose medium dose, 7AU 51 included in full analysis for safety	51 first high dose, 35AU 51 included in full analysis for safety

1 COVID-19 1Adverse Event (haematuria)	1 Pregnancy

51 second low dose vaccine, 3AU 51 included in per-protocol immunogenicity analysis	49 second dose medium dose 7AU 49 included in per-protocol immunogenicity analysis	50 second high dose 35AU 50 included in per-protocol immunogenicity analysis

SAR-CoV-2 N-protein antibody positive

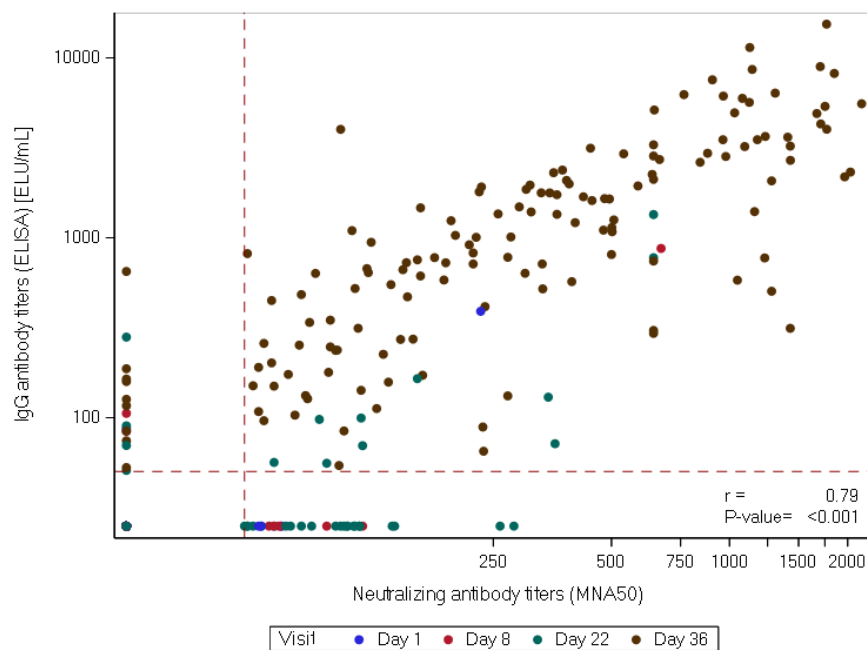| 51 second low dose vaccine, 3AU 51 included in per-protocol immunogenicity analysis	48 second dose medium dose 7AU 48 included in per-protocol immunogenicity analysis	50 second high dose 35AU 50 included in per-protocol immunogenicity analysis

Figure 1 Study Consort Diagram

Figure 2 Correlation between neutralizing antibody titres ND50 and IgG antibody titres. Red

dotted lines present the limit of detection for ELISA (50.3 ELU/mL) and MNA (ND50=58). (r)

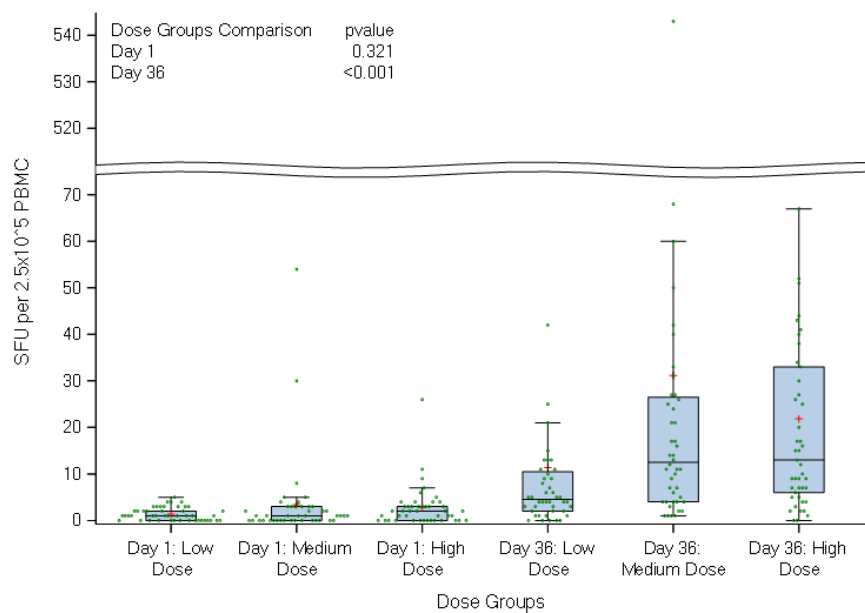represents Pearson correlation coefficient

Figure 3 T-cell ELISpot responses to full sequence SARS-CoV-2 Spike protein. Boxplots show median, lower quartile and upper quartile; the horizontal line within each bar is the median and red + sign represents the mean value for each group. Green dots represent the measured values of SFU per 2.5x10^5 PBMC within each group

**Valneva phase 1 trial group

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Supplementary material
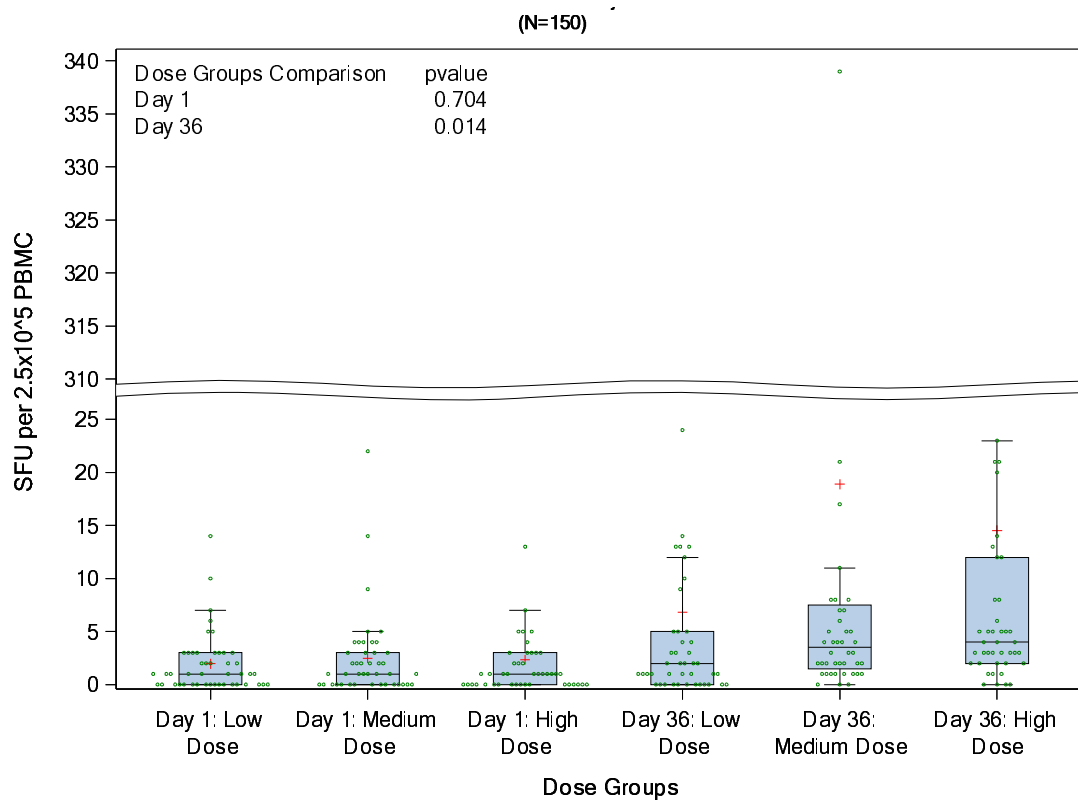
Supplementary figure 1. T-cell ELISpot responses to SARS-CoV-2 Membrane protein. Boxplots show median, lower quartile and upper quartile; the horizontal line within each bar is the median and red + sign represents the mean value for each group. Green dots represent the measured values of SFU per 2.5x10^5 PBMC within each group
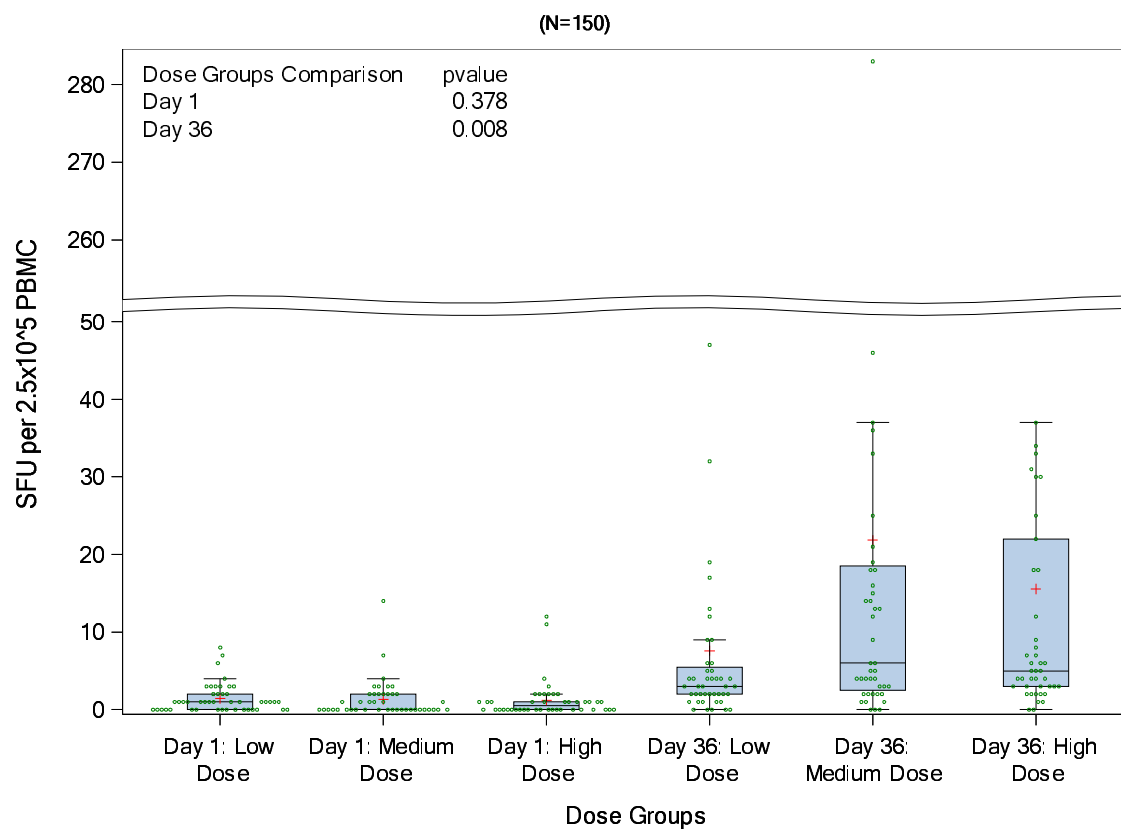
Supplementary figure 2. T-cell ELISpot responses to SARS-CoV-2 Nucleocapsid protein. Boxplots show median, lower quartile and upper quartile; the horizontal line within each bar is the median and red + sign represents the mean value for each group. Green dots represent the measured values of SFU per 2.5x10^5 PBMC within each group

Appendix A Study Protocol