Research Paper

Safety and Immunogenicity of a DNA SARS-CoV-2 vaccine (ZyCoV-D): Results of an open-label, non-randomized phase I part of phase I/II clinical study by intradermal route in healthy subjects in India

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ABSTRACT

Background: ZyCoV-D is a DNA vaccine candidate, which comprises a plasmid DNA carrying spike-S gene of SARS-CoV-2 virus along with gene coding for signal peptide. The spike(S) region includes the receptor-binding domain (RBD), which binds to the human angiotensin converting Enzyme (ACE)-2 receptor and mediates the entry of virus inside the cell.
Methods: We conducted a single-center, open-label, non-randomized, Phase 1 trial in India between July 2020 and October 2020. Healthy adults aged between 18 and 55 years were sequentially enrolled and allocated to one of four treatment arms in a dose escalation manner. Three doses of vaccine were administered 28 days apart and each subject was followed up for 28 days post third dose to evaluate safety and immunogenicity.
Findings: Out of 126 individuals screened for eligibility. Forty-eight subjects (mean age 34·9 years) were enrolled and vaccinated in the Phase 1 study Overall, 12/48 (25%) subjects reported at least one AE (i.e. combined solicited and unsolicited) during the study. There were no deaths or serious adverse events reported in Phase 1 of the study. The proportion of subjects who seroconverted based on IgG titers on day 84 was 4/11 (36·36%), 4/12 (33·33%), 10/10 (100·00%) and 8/10 (80·00%) in the treatment Arm 1 (1 mg: Needle), Arm 2 (1 mg: NFIS), Arm 3 (2 mg: Needle) and Arm 4 (2 mg: NFIS), respectively.
Interpretation: ZyCoV-D vaccine is found to be safe, well-tolerated and immunogenic in the Phase 1 trial. Our findings suggest that the DNA vaccine warrants further investigation.

1. Introduction

Severe Acute Respiratory Syndrome coronavirus 2019 (COVID-19), emerged in December 2019 in Wuhan, China [1]. A novel coronavirus was identified as the etiologic agent in January 2020. The genetic sequence of the virus became available (MN908947.3) in January 2020. Within months of emergence, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections and the resulting disease, COVID-19, spread worldwide. On 11 March 2020, the World Health Organization (WHO) declared the COVID-19 outbreak a pandemic [2].

COVID-19 disease is rapidly transmitted from human to human, with influenza-like symptoms ranging from mild disease to severe disease and multi-organ failure, eventually resulting in death, especially in aged patients with co-morbid conditions [3,4]. Vaccines are considered to the most effective treatment to control the pandemic and help to restore the global economy [5,6]. There are currently more than 63 COVID-19 candidate vaccines undergoing clinical trials and more than 172 COVID-19 candidate vaccines undergoing pre-clinical development worldwide, including mRNA vaccines, replicating or non-replicating viral vectored vaccines, DNA vaccines,

Research in context

Evidence before this study

We searched PubMed on March 23, 2021, using the search terms "COVID-19″, "SARS-CoV-2″, "vaccine", and "clinical trial". Cadila Healthcare Limited, India developed a candidate vaccine ZyCoV-D comprising of a DNA plasmid vector carrying the gene encoding the spike protein (S) of the SARS-CoV-2 virus. Preliminary animal study demonstrates that the candidate DNA vaccine induces antibody response including neutralizing antibodies against SARSCoV-2 and provided Th-1 response as evidenced by elevated IFN-γ levels.

Added value of this study

This first-in-human trial showed that the DNA vaccine was tolerable and immunogenic in healthy adults. The DNA vaccine candidate induces antibody response against SARS-CoV-2 spike (S) protein, following immunization with three doses administered 28 days apart. Neutralizing antibody response was also demonstrated against wild type SARS-CoV-2 strain, which may play a substantial role in viral clearance and mitigation of human clinical disease. This study has also evaluated safety and immunogenicity of DNA vaccine administered by two different methods of administration.

Implications of all the available evidence

Implications of all the available evidence Many vaccine candidates are in rapid development, including recombinant-protein based vaccines, replicating or non-replicating viral vector-based vaccines, DNA vaccines, and mRNA vaccines (which mostly have focused on the spike glycoprotein or receptor binding domain), live attenuated vaccines, and inactivated virus vaccines. Our findings indicate that DNA vaccine is safe and immunogenic in healthy adults.

autologous dendritic cell-based vaccine, and inactive virus vaccines [7]. The results of the Phase 1 and 2 trials of several vaccines, such as a chimpanzee adenovirus-vectored vaccine, recombinant adenovirus type-5 (Ad5)−vectored vaccine, inactivated vaccines and mRNA vaccines, have been published. The results of few phase 3 trials have also been published now [8,9].

In December 2020, Pfizer Inc. and BioNTech SE received a temporary authorization for emergency use of COVID-19 mRNA vaccine against COVID-19 from the USFDA. The Regulatory Agencies approved this vaccine in the UK, Canada, Saudi Arabia and Bahrain as well [10]. Moderna, Pfizer, Johnson & Johnson (USA and EU-approved vaccines) as well as AstraZeneca and others (EU-approved vaccines) also received a temporary authorization for emergency use. On 16th January, 2021, the first COVID-19 vaccine received a temporary authorization for emergency use by the Drug Controller General of India (DCGI) in India and many more remain in development.

As of May 16, 2021, SARS-CoV-2 had infected more than 162 million people and killed more than 3.3 million since the start of the pandemic worldwide [11]. The number of reported SARS-CoV-2 cases in India till May 19, 2021 is also on an increase with ~25 million confirmed cases and ~ 283,248 deaths [12]. The worldwide impact of this pandemic on human society calls for the rapid development of safe and effective therapeutics and vaccines.

Here, we report the safety and immunogenicity of DNA based SARS-CoV-2 vaccine data from the Phase 1 clinical trial of an ongoing, Phase 1/2 clinical study, which commenced in July 2020 to evaluate the impact of ZyCoV-D vaccine in preventing Covid-19 in healthy adult subjects, 18−55 years of age. The data includes evaluation of the 1 mg and 2 mg dose levels of ZyCoV-D vaccinated healthy adult subjects. Collection of phase 2 data on vaccine immunogenicity and the durability of the immune response following vaccination is ongoing, and those data are not reported here.

We have developed a DNA vaccine candidate for prevention of COVID-19. It is comprised of a plasmid DNA carrying spike-S gene of SARS-CoV-2 virus along with gene coding for signal peptide. The spike(S) region includes the receptor binding domain (RBD), which binds to the human angiotensin converting Enzyme (ACE)-2 receptor and mediates the entry of virus inside the cell. The DNA construct was produced on large scale by transformation in E. coli [13]. The immunogenicity potential of the plasmid DNA has been evaluated in mice, guinea pig, and rabbit models by intradermal route at 25, 100 and 500 μg dose. Preliminary studies have demonstrated that the DNA vaccine induces antibody response including neutralizing antibodies (NAB) against SARS-CoV-2 and also provides Th-1 response as evidenced by elevated IFN-γ levels [13].

In fact, a similar approach has already been used in the past for development of Middle East Respiratory Syndrome (MERS) and SARS coronavirus vaccines [14,15]. The MERS DNA vaccine was found to be well-tolerated in humans with a seroconversion rate of 94% in vaccinated volunteers, whereas, the SARS DNA vaccine induced antibody response in 80% subjects. Based on the earlier published literature of similar vaccines, the expected human dose of 2019-nCoV vaccine by intradermal administration will be 1 mg or 2 mg of the 2019-nCoV DNA vaccine candidate.

2. Methods

2.1. Study design and participants

We conducted a single-center Phase 1 trial of the DNA plasmid spike protein COVID-19 vaccine candidate at a Clinical Unit of Zydus Research center, Cadila Healthcare Limited in Ahmedabad, Gujarat, India.

This trial was initiated after obtaining the approvals of the Ethics Committee (EC) and DCGI (dated 08 July 2020) and registering the trial with the Clinical Trial Registry of India (CTRI) (Identifier: CTRI/2020/07/026352). The study was performed in accordance with the Declaration of Helsinki, Good Clinical Practice and applicable local regulations. An independent data safety monitoring board was established before the start of the study to provide oversight of the safety data during the study. The authors had full access to all the data in the study.

Eligible participants were healthy adults aged between 18 and 55 years; body weight >50 kg; body-mass index of between 18.5 and 29.9 kg/m^2. For inclusion in the trial, participants needed to be able to understand the content of informed consent and be willing to sign the informed consent document; and be able and willing to complete all the scheduled study visits. Exclusion criteria included SARS-CoV-2 infection, confirmed by presence of serum-specific antibody against SARS-Co-V-2 detected by enzyme-linked immunosorbent assay (ELISA) or chemiluminescence technology; positive results for COVID-19 as detected by qualitative reverse transcription polymerase chain reaction; history of SARS/ MERS infection; history of contact with a confirmed active SARS-CoV-2 positive patient within 14 days; participation in other clinical study of a SARS-CoV-2 candidate vaccine. Pregnant or breastfeeding women were also excluded. A comprehensive list of eligibility criteria is provided (Supplementary Table S1). Written informed consent was obtained from each participant before screening for eligibility.

2.2. Vaccine

The ZyCoV-D vaccine was developed by Cadila Healthcare Limited, Ahmedabad, India. The DNA vaccine candidate against SARS-CoV-2 is

comprised of a DNA plasmid Vector pVAX1 carrying gene expressing spike-S protein of SARS-CoV-2 and IgE signal peptide. The spike gene region was selected from submitted Wuhan Hu-1 isolate (Genebank Accession No. MN908947.3). For generation of the SARS-CoV-2 DNA vaccine construct pVAX-1 plasmid vector was used. Chemically synthesized Spike regions and signal peptide gene were inserted into pVAX-1 plasmid DNA vaccine vector. Following the receipt of the plasmid DNA constructs, transformations of the construct were carried out in DH5-alpha^TM chemically competent cells. The DH5-alpha E coli carrying the plasmid DNA was further propagated for large scale production in manufacturing suite approved by National Regulatory Authority under current Good Manufacturing Practice conditions. Each 0.5 mL of ZyCoV-D vaccine contains -5 mg of DNA plasmid with spike protein gene region insert from SARS-CoV-2 Virus suspended in phosphate buffer saline.

2.3. Procedures

Participants were recruited and followed up with according to treatment arms. Participants were allocated to treatment arms sequentially in a non-randomized, open label, dose escalation manner (i.e. the first 12 subjects were allocated to treatment arm 1 [1 mg: Needle]; the next 12 subjects were allocated to treatment arm 2 [1 mg; Needle-Free Injection System {NFIS}] once safety was established in the previous treatment arm; same was followed for treatment arm 3 [2 mg; Needle] and treatment arm 4 [2 mg; NFIS]). Participants were immunized intradermally with three doses of vaccine (1 mg: Needle or NFIS; 2 mg: Needle or NFIS) four weeks apart via syringe and needle or NFIS on days 0, 28 and 56. Follow-up visits were scheduled after each vaccination until day 84 (End-of-study). In this study, Pharmajet Tropis® device as NFIS was used for intradermal administration of the vaccine. Participants were monitored in the intensive observation unit for 24 h post the first dose of vaccine and 4 h post the second and third dose of vaccine for solicited adverse reactions (injection site pain, redness, swelling and itching). Close monitoring in terms of frequent vital signs and electrocardiogram (ECG) assessments were done before and after each vaccine dose. Subjects were also provided a diary card to record any solicited systemic symptoms (fever, headache, fatigue, vomiting, diarrhea, nausea, arthralgia, and muscle pain) and local adverse events (AEs) for 7 days post each vaccine dose and any other unsolicited AEs within 28 days post each dose. Serious AEs self-reported by participants were documented throughout the study.

Adverse events were self-reported by the participants, but verified by investigators throughout the study after vaccination. Adverse events were graded according to a standard toxicity grading scale [16]. Laboratory safety tests including hematology, biochemistry, urinalysis and serology were conducted as per the protocol (Appendix 1) to assess any toxic effects post-vaccination. Blood samples were taken from participants as per the protocol for the immunogenicity assessment. The follow-ups were scheduled at days 70 and 84 (end-of-study) post vaccination for safety and immunogenicity assessment.

2.4. Assessment of binding antibody (IgG and neutralizing antibody) and cellular response

For Phase 1 part of the study, immunogenicity assessment for serum IgG by ELISA, was done at baseline, day 28, day 42, day 56, day 70 and day 84. Neutralizing antibody titres and cellular response were also assessed at baseline, day 28, day 56 and day 84.

An indirect ELISA was used to measure anti-S1 SARS CoV-2 IgG antibodies present in the human sera samples post vaccination with ZyCoV-D vaccine. We used antigen from Acro Biosystems. The antigen from the same manufacturer was also used by Innovio in their ELISA assay [17]. We used reference standard from National Institute for Biological Standards and Control (NIBSC) which is a WHO reference laboratory. We obtained research reagent for anti-SARS-CoV-2 Ab NIBSC code 20/130 and against this we defined unitage as ELISA Unit (EU). Plaque Reduction Neutralization Test (PRNT) was used for estimation of NAB titer in human serum samples against anti SARS CoV-2 virus. The SARS-CoV-2 virus (8004/IND/2020/PUNE), Accession number − MT416726 was used for PRNT assay.

Cell-mediated responses were assessed using IFN-γ ELISPOT assay in separated peripheral blood mononuclear cells (PBMCs). Serum samples from vaccinated subjects were also analyzed for the cytokines levels (IFN-γ, IL-2, IL-6, IL-4, IL-10, TNF alpha, Th-17A) using MILLIPLEX® MAP multiplex magnetic bead-based antibody detection kits. Details regarding the methodology of these tests are provided in supplementary material.

2.5. Outcomes

The primary endpoint was the overall incidence and severity of adverse reactions within 7 days after each of the vaccination and AEs within 28 days across the treatment groups were also analyzed as safety endpoints.

The secondary endpoints included seroconversion rate based on IgG antibodies against S1 antigen (by ELISA), NAB titers and IFN-γ cellular immune responses after 3 doses of vaccine. Seroconversion was defined as antibody-negative subjects at baseline who become antibody-positive after vaccination, and subjects having antibody titre at baseline who have four fold rise in antibody titre after vaccination.

2.6. Statistical analysis

The sample size was not determined on the basis of statistical power calculations. Sample size was based on non-probability sampling method. However, a minimum sample size of 48 participants for this vaccine trial has been selected. We assessed the incidence and severity of participants' adverse reactions post vaccination and compared safety profiles across the dose groups. The antibodies against SARS-CoV-2 were presented as geometric mean titers with 95% confidence intervals (CIs) and the cellular responses were shown as a proportion of positive responders. We used the pearson chi-square test to analyze categorical data, ANOVA to analyze the log transformed antibody titers. Hypothesis testing was two-sided with an α value of 0.05. Statistical analyses were done by a statistician using SAS (version 9.4).

Geometric mean titres (GMTs) was calculated as: anti-Ln(mean [Ln Xi]) where Xi was the assay result for subject i. 95% CIs of GMTs were calculated assuming log normal distribution.

Geometric Mean Fold Rise (GMFR) were calculated as: GMFR = anti-Ln (mean [Ln Yi/ Bi]) where Yi was the post dose assay result for subject *i*; and Bi was the baseline assay result for subject *i*. Baselines were taken as Day 0 assay results.

2.7. Role of the funding source
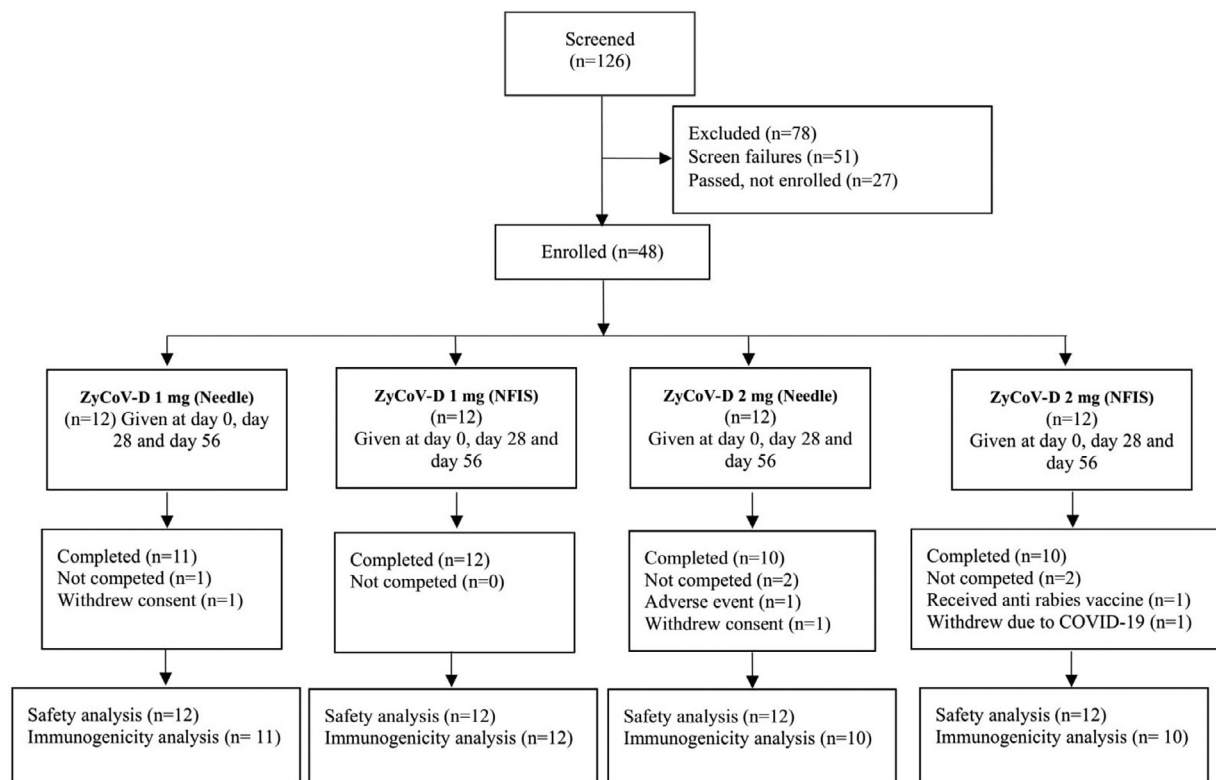
Fig. 1. Subject allocation and participation in the completion of the study. Consort chart explaining subject participation (*n* = number of subject) in different treatment arms NIFS = Needle-free injection system.

3. Results

3.1. Study population demographics

Between July 2020 and October 2020, 126 participants were screened, 51 subjects failed in screening, 27 subjects passed but not enrolled in the study and a total of 48 subjects were enrolled into and vaccinated in the Phase 1 study. A total of 43 (89·0%) participants completed the study and 5 subjects were discontinued from the study (Fig. 1). All of the 48 participants (100%) were Asian male healthy subjects. Baseline demographics (age, height, weight and BMI) were well-balanced among the 4 treatment arms. Overall, the mean (SD) age was 34·9 (7·18) years and the mean BMI (SD) was 24·28 ± 3·0 kg/m^2. The patient disposition, baseline and demographic characteristics are provided in Table 1.

3.2. Vaccine safety and tolerability

A total of 48 subjects were vaccinated with the first dose of vaccine, 45 subjects were vaccinated with the second dose of vaccine (except 1 subject in arm 1 and 2 subjects in arm 4 who received only one dose) and 43 subjects were vaccinated with the third dose of vaccine (except 2 subjects in arm 3 who received only two doses). A total of 43 subjects completed the study.

Of the five subjects who discontinued, two subjects were discontinued because of withdrawal of consent (1 subject in each arm 1 and 3); one subject in arm 3 did not receive the third dose of vaccine due to an ongoing unsolicited adverse event (typhoid fever) that occurred 14 days after the second vaccination which was considered not related with vaccination; one subject in arm 4 was discontinued due to an ongoing anti-rabies vaccination for dog bite reported 7 days after the first vaccination; and one subject in arm 4 was withdrawn due to asymptomatic positive COVID-19 rapid antigen detection test, 27 days after the first vaccination.

There were no deaths or SAEs reported in Phase 1 of the study. Overall, 12/48 (25%) subjects reported at least one AE (i.e. combined solicited and unsolicited) during the study. The number of subjects with at least one solicited adverse event and at least one unsolicited adverse event was 7 (14·58%) subjects and 6 (12·5%) subjects, respectively (Figs. 2 and 3).

The number of subjects with solicited AEs across all four treatment arms were similar [i.e. 2 subjects in each Arm 1 (1 mg: Needle), Arm 2 (1 mg: NFIS) and Arm 4 (2 mg: NFIS); 1 subject in Arm 3 (2 mg: Needle)]. Overall, all solicited AEs reported were mild to moderate in severity, related with study vaccination and resolved with or without medication. The majority of solicited AEs were reported after the first dose of vaccine (i.e. 6 subjects, 12·5%) compared to the second dose (0%) and the third dose of vaccination (1 subject, 2·08%). Most solicited AEs were mild in severity (6 subjects, 12·5%) except 1 subject (2·08%) who reported an adverse event of moderate severity. No subject was discontinued from the study because of a solicited adverse event. The reported solicited AEs were injection site pain (3 subjects), injection site pruritus (1 subject), pyrexia (1 subject), arthralgia (1 subject) and diarrhea (1 subject).

There were no abnormal laboratory values that were deemed clinically significant except proteinuria (1 subject on day 14), considered possibly related to study drug and low WBC count (one subject on day 56), considered not related to the study drug by the investigator throughout the study period. There were no clinically significant changes reported in vital signs and 12-lead ECG evaluated during the monitoring period after vaccination of each dose as well as follow-up visits till day 84. For all the physical examinations performed, no major abnormal findings were reported till day 84.

3.3. Immune responses

Seroconversion was defined as antibody negative subjects at baseline who become antibody positive after vaccination and subjects

Table 1
Disposition, Baseline and Demographics Characteristics − Safety Population.

Parameters/Statistic	ZyCoV-D 1 mg (Needle) (N = 12)	ZyCoV-D 1 mg (NFIS) (N = 12)	ZyCoV-D 2 mg (Needle) (N = 12)	ZyCoV-D 2 mg (NFIS) (N = 12)	Overall (N = 48)
Disposition					
All subjects, n (%)	12 (100)	12 (100)	12 (100)	12 (100)	48 (100)
Subjects who completed the study, n (%)	11 (91.67)	12 (100)	10 (83.33)	10 (83.33)	43 (89.58)
Subjects discontinued from the study, n (%)	1 (8.33)	0 (0)	2 (16.67)	2 (16.67)	5 (10.42)
Demographics					
Age (Years)					
Mean ± SD	35.4 ± 6.56	31.8 ± 7.44	35.1 ± 6.95	37.2 ± 7.53	34.9 ± 7.18
Median (Range)	36.5 (27, 45)	31.5 (22, 48)	36.0 (20, 45)	36.5 (26, 48)	35.5 (20, 48)
Sex, n (%)					
Male	12 (25%)	12 (25%)	12 (25%)	12 (25%)	48 (100%)
Height (cm)					
Mean ± SD	168.5 ± 4.08	166.9 ± 4.01	165.8 ± 4.53	169.3 ± 5.83	167.6 ± 4.72
Median (Range)	170.0 (161, 173)	167.0 (159, 173)	165.5 (159, 173)	170.0 (159, 181)	168.0 (159, 181)
Weight (kg)					
Mean ± SD	68.52 ± 7.878	65.32 ± 7.751	70.32 ± 8.562	67.97 ± 11.052	68.03 ± 8.810
Median (Range)	67.80 (57.6, 82.8)	66.65 (54.2, 76.2)	70.30 (56.8, 85.7)	66.60 (54.7, 87.5)	67.95 (54.2, 87.5)
BMI (kg/m^2)					
Mean ± SD	24.178 ± 3.0745	23.416 ± 2.3624	25.593 ± 3.0485	23.720 ± 3.5076	24.227 ± 3.0470
Median (Range)	24.355 (19.98, 29.71)	23.860 (18.98, 26.40)	26.690 (18.98, 29.31)	23.805 (18.71, 29.58)	24.320 (18.71, 29.71)

Abbreviation(s): N = number of subjects in respective treatment arm; n = number of subjects in specified category; NFIS = Needle Free Injection System; BMI = body mass index; SD = standard deviation. Note: Percentages are based on the number of subjects in the specified treatment arm.

having antibody titre at baseline who have four-fold rise in antibody titre after vaccination.

As mentioned in the method section, we used NIBSC reference standard sera in our ELISA assay. Using this standard we established the standard curve range from 1.41 EU as below limit of (BLQ) to 45.23 EU as upper limit of quantification (ULQ). We also tested panel of negative pre-COVID-19 sera sample during assay validation and samples were below the BLQ value of 1.41EU. NIBSC standard was also used in ELISA assay performed by Oxford group for their

immunogenicity evaluation of ChADOx-1 SARS-CoV-2 vaccine candidate [18]. The proportion of subjects who seroconverted based on IgG titers on day 84 (i.e. 28 days after third vaccine doses) was 4 (36·36%), 4 (33·33%), 10 (100·00%) and 8 (80·00%) in the treatment Arm 1 (1 mg: Needle), Arm 2 (1 mg: NFIS), Arm 3 (2 mg: Needle) and Arm 4 (2 mg: NFIS), respectively. This suggests a higher seroconversion rate with the 2 mg vaccine dose, irrespective of method of delivery, compared to the 1 mg vaccine dose. When Arm 1 (1 mg: Needle), and Arm 4 (2 mg: NFIS), seroconversion rates were compared for day
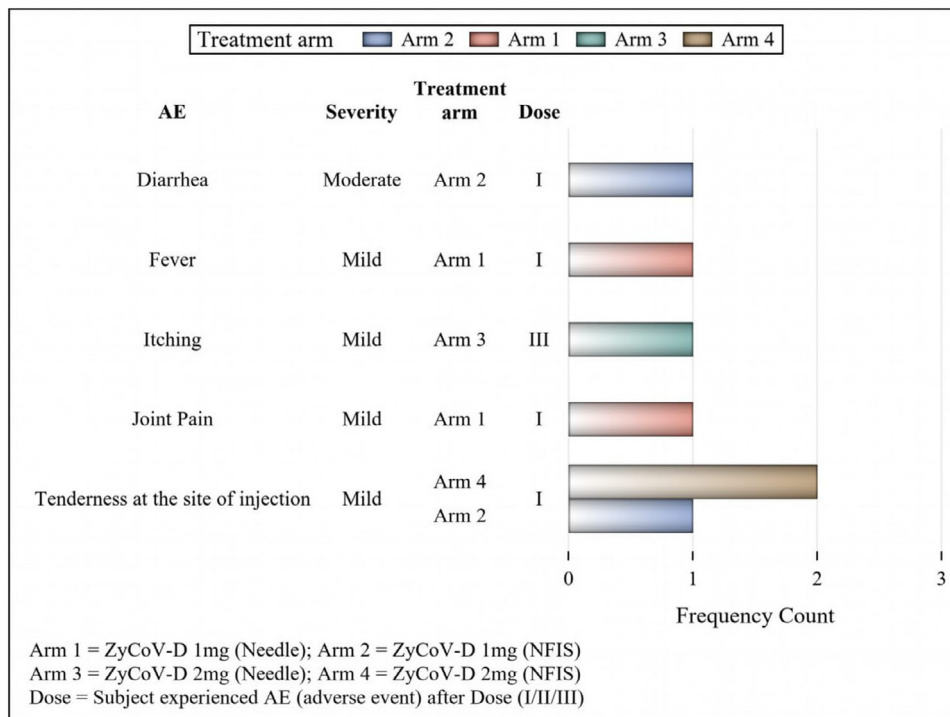
Fig. 2. Solicited (local and systemic) adverse events reported within seven days after administration of each dose of vaccine. Adverse events were graded according to the common terminology criteria for adverse events (CTCAE) scale.
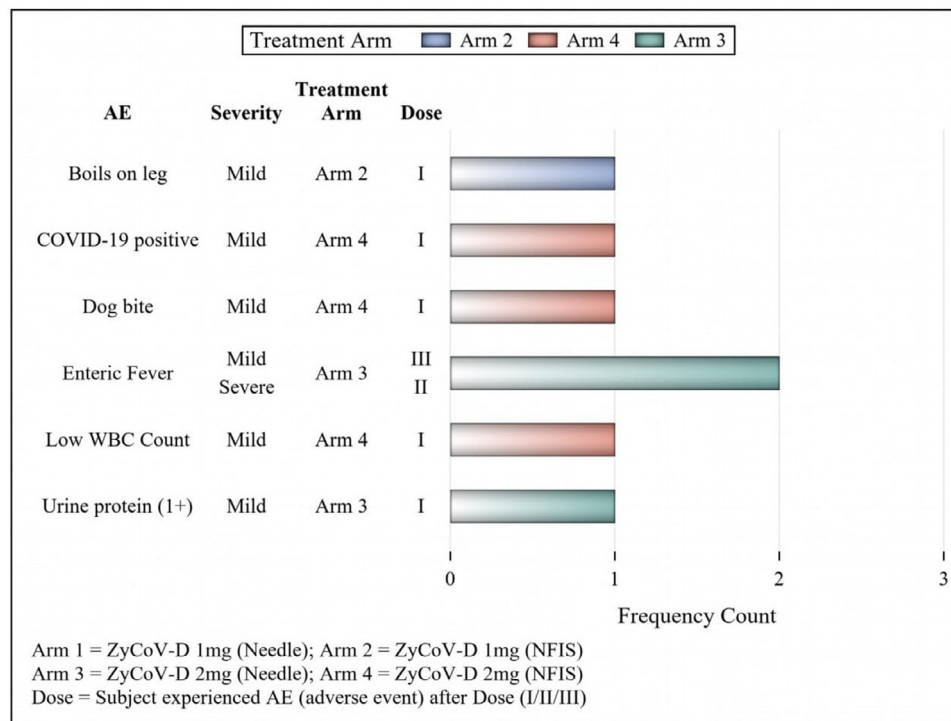
Fig. 3. Unsolicited (local and systemic) adverse events reported after seven days of each dose of vaccine. Adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) scale.

28, day 42, day 56, day 70 and day 84, a statistical significant (p value = 0.0019) difference was found for day 70. Similarly for Arm 2 (1 mg: NFIS) vs Arm 4 (2 mg: NFIS), statistical significant (p value = 0.0427) difference was found for day 70 and day 84 (Supplementary Table S2).

The proportion of subjects who achieved seroconversion based on IgG on day 28, day 42, day 56, day 70 and day 84 is mentioned in Table 2. We have also included NAB titers of convalescent sera sample from individuals recovered after SARS-CoV-2 infection (Fig. 4).

The proportion of subjects getting seroconverted based on NAB titers on day 84 was 02 (18.18%), 02 (16.67%), 05 (50.00%) and 08 (80.00%) in the treatment Arm 1 (1 mg: Needle), Arm 2 (1 mg: NFIS), Arm 3 (2 mg: Needle) and Arm 4 (2 mg: NIFS) respectively. The proportion of subjects getting seroconverted based on NAB titers on day 56 was lower i.e. 00 (00.00%), 02 (16.67%), 02 (20.00%) and 01 (10.00%) in the treatment Arm 1 (1 mg: Needle), Arm 2 (1 mg: NFIS), Arm 3 (2 mg: Needle) and Arm 4 (2 mg: NIFS) respectively. When Arm 1 (1 mg: Needle), and Arm 4 (2 mg: NFIS), seroconversion rates based on NAB titers were compared for day 56 and day 84, a

statistical significant (p value = 0.0089) difference was found for day 84. Similarly for Arm 2 (1 mg: NFIS) vs Arm 4 (2 mg: NFIS), statistical significant (p value = 0.0083) difference was found for day 84 (Supplementary Table S3).

Geometric mean titer of IgG (EU) on day 56 (28 days after two doses of vaccine) was 34·75 and 17·46 in the treatment Arm 3 (2 mg: Needle) and Arm 4 (2 mg: NFIS), respectively, which increased to 1019·61 and 720·25 in the treatment Arm 3 (2 mg: Needle) and Arm 4 (2 mg: NFIS) respectively on day 70 (14 days after three doses of vaccine) and remained stable at 748·46 and 884·04 in the treatment Arm 3 (2 mg: Needle) and Arm 4 (2 mg: NFIS) respectively on day 84 (28 days after three doses of vaccine) See, Table 3 and Fig. 5. When Arm 1 (1 mg: Needle), and Arm 4 (2 mg: NFIS), Geometric mean titer of IgG (EU) were compared for day 28, day 42, day 56, day 70, and day 84 a statistical significant (p value = 0.0006) difference was found for day 70 and day 84 (p value = 0.0027). Similarly for Arm 2 (1 mg: NFIS) vs Arm 4 (2 mg: NFIS), statistical significant (p value = 0.0376) difference was found for day 70 and p-values= 0.0259 for day 84 (Supplementary Table S4).

Table 2
Summary and Comparison of Seroconversion of IgG at day 28, 42, 56, 70 and 84.

Time point	Seroconversion	ZyCoV-D 1 mg (Needle) (N = 11)	ZyCoV-D 1 mg (NFIS) (N = 12)	ZyCoV-D 2 mg (Needle) (N = 10)	ZyCoV-D 2 mg (NFIS) (N = 10)
Day-28, n (%)	No	9 (81.82)	9 (75.00)	6 (60.00)	7 (70.00)
	Yes	2 (18.18)	3 (25.00)	4 (40.00)	3 (30.00)
Day-42, n (%)	No	10 (90.91)	9 (75.00)	7 (70.00)	7 (70.00)
	Yes	1 (9.09)	3 (25.00)	3 (30.00)	3 (30.00)
Day-56, n (%)	No	9 (81.82)	7 (58.33)	4 (40.00)	6 (60.00)
	Yes	2 (18.18)	5 (41.67)	6 (60.00)	4 (40.00)
Day-70, n (%)	No	10 (90.91)	8 (66.67)	0 (0.00)	2 (20.00)
	Yes	1 (9.09)	4 (33.33)	10 (100.0)	8 (80.00)
Day-84, n (%)	No	7 (63.64)	8 (66.67)	0 (0.00)	2 (20.00)
	Yes	4 (36.36)	4 (33.33)	10 (100.0)	8 (80.00)

Abbreviation(s): N = number of subjects in respective treatment arm; n = number of subjects in specified category; NFIS = Needle Free Injection System.
Seroconversion defined as a positive antibody response as at least a four-fold increase in post-vaccination titer from baseline.
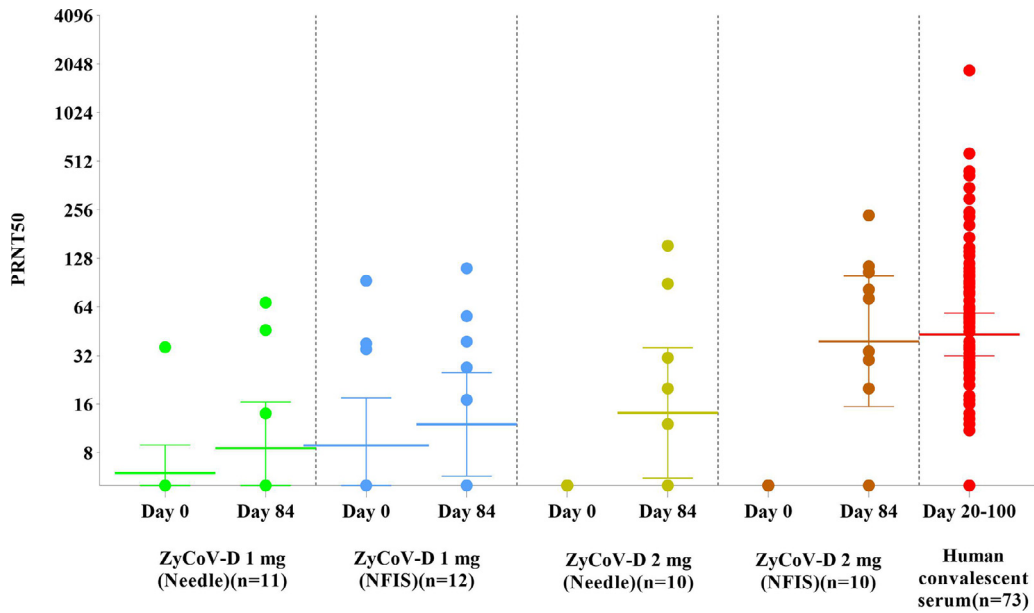
Fig. 4. Neutralising antibody titres with error bars, representing geometric mean (95%CI) at baseline (Day 0) and Day 84 with comparative NAB of convalescent serum.

Geometric mean titer of NAB on day 84 (28 days after three doses of vaccine) was 39.17 in the treatment Arm 4 (2 mg: NFIS). Geometric mean titer and fold rise of NAB are presented in Table 4.

When Arm 1 (1 mg: Needle), and Arm 4 (2 mg: NFIS), Geometric mean titer of NAB were compared for day 56, and day 84 a statistical significant (p value = 0.0055) difference was found for day 84. Similarly for Arm 2 (1 mg: NFIS) vs Arm 4 (2 mg: NFIS), statistical significant (p value = 0.0251) difference was found for day 84 (Supplementary Table S5).

Seroconversion rates of IgG with 2 mg needle (Arm 3) and 2 mg NFIS (Arm 4) on day 56 and day 84 were observed higher than 1 mg needle and 1 mg NFIS (arm 1 and 2). Seroconversion rates of NAB with 2 mg NFIS (Arm 4) on day 84 were observed higher than 1 mg needle, 1 mg NFIS and 2 mg needle (Table 5). The seroconversion rate (defined as subjects sero-negative at baseline becoming sero-positive post vaccination and four fold rise in antibody titres post vaccination in subjects sero-positive at baseline)) based on humoral responses measured by ELISA were observed in 100% and 80% of the participants who received three doses of 2 mg vaccine either via needle and syringe or NFIS device respectively. The seroconversion rate based on NAB, measured by live virus neutralization assay, was seen in 50% (05/10) and 80% (08/10) of participants who received three doses of 2 mg vaccine either via needle and syringe or NFIS device respectively.

3.4. Cell-mediated responses

In our study, ZyCoV-D vaccine, when administered intradermally via NFIS at 2 mg dose, showed peak cellular response in terms of IFN-γ ELISPOT assay at Day 56 with 41.5 spot forming cells (SFC) per million PBMC's and was maintained till Day 84 with median 45.5 SFC per million PBMC's. A similar trend was observed with 1 mg NFIS arm with Day 84 median 73 SFC per million PBMC's. ZyCoV-D vaccine when administered by conventional syringe and needle showed some response in IFN-γ ELISPOT assay at Day 56 which declined on Day 84 after reaching the peak on day 56 (Fig. 6).

In our study, there were no significant changes observed in cytokine levels like. IFN-γ, IL-2, IL-6, IL-4, IL-10, TNF alpha, Th-17A analysed by Luminex in all four-treatment arms throughout the study compared to baseline.

4. Discussion

We report the findings from Phase 1 part of clinical trial on the safety, tolerability and immunogenicity of ZyCoV-D, a SARS-CoV-2 DNA vaccine encoding the spike protein. This first-in-human Phase 1 study of ZyCoV-D DNA vaccine was carried out in an intensive observational unit with frequent monitoring of vital signs and ECGs for at least 24 h post administration of the first

Table 3
GMTs of IgG at day 28, 42, 56, 70 and 84 post dose − sero negative subjects only.

Time Point	Statistics	ZyCoV-D 1 mg (Needle) (N = 11)	ZyCoV-D 1 mg (NFIS) (N = 12)	ZyCoV-D 2 mg (Needle) (N = 10)	ZyCoV-D 2 mg (NFIS) (N = 10)
Day-0	GMT(95% C.I)	15.82 (4.61, 54.25)	26.73 (7.45, 95.89)	7.00 (7.00, 7.00)	7.00 (7.00, 7.00)
Day-28	GMT(95% C.I)	26.92 (6.46, 112.12)	66.39 (14.11, 312.27)	11.42 (6.80, 19.17)	14.15 (4.79, 41.79)
	GMFR(95% C.I)	1.70 (0.94, 3.07)	2.48 (0.93, 6.67)	1.63 (0.97, 2.74)	2.02 (0.68, 5.97)
Day-42	GMT(95% C.I)	20.41 (5.35, 77.84)	55.67 (12.48, 248.37)	11.01 (6.29, 19.26)	15.60 (4.57, 53.20)
	GMFR(95% C.I)	1.29 (0.86, 1.93)	2.08 (0.83, 5.26)	1.57 (0.90, 2.75)	2.23 (0.65, 7.60)
Day-56	GMT(95% C.I)	23.37 (6.34, 86.22)	115.91 (23.41, 573.98)	34.75 (8.11, 148.89)	17.46 (4.81, 63.32)
	GMFR(95% C.I)	1.48 (0.91, 2.40)	4.34 (1.14, 16.44)	4.96 (1.16, 21.27)	2.49 (0.69, 9.05)
Day-70	GMT(95% C.I)	20.10 (5.40, 74.77)	94.84 (18.49, 486.47)	1019.61 (326.04, 3188.54)	720.25 (123.28, 4207.96)
	GMFR(95% C.I)	1.27 (0.85, 1.91)	3.55 (0.93, 13.56)	145.66 (46.58, 455.51)	102.89 (17.61, 601.14)
Day-84	GMT(95% C.I)	38.84 (9.80, 154.00)	96.49 (19.65, 473.89)	748.46 (237.75, 2356.25)	884.04 (138.69, 5635.19)
	GMFR(95% C.I)	2.46 (0.92, 6.59)	3.61 (0.94, 13.93)	106.92 (33.96, 336.61)	126.29 (19.81, 805.03)

Abbreviation(s): N = number of subjects in respective treatment arm; n = number of subjects in specified category; NFIS = Needle Free Injection System.
Seroconversion defined as a positive antibody response as at least a four-fold increase in post-vaccination titer from baseline.
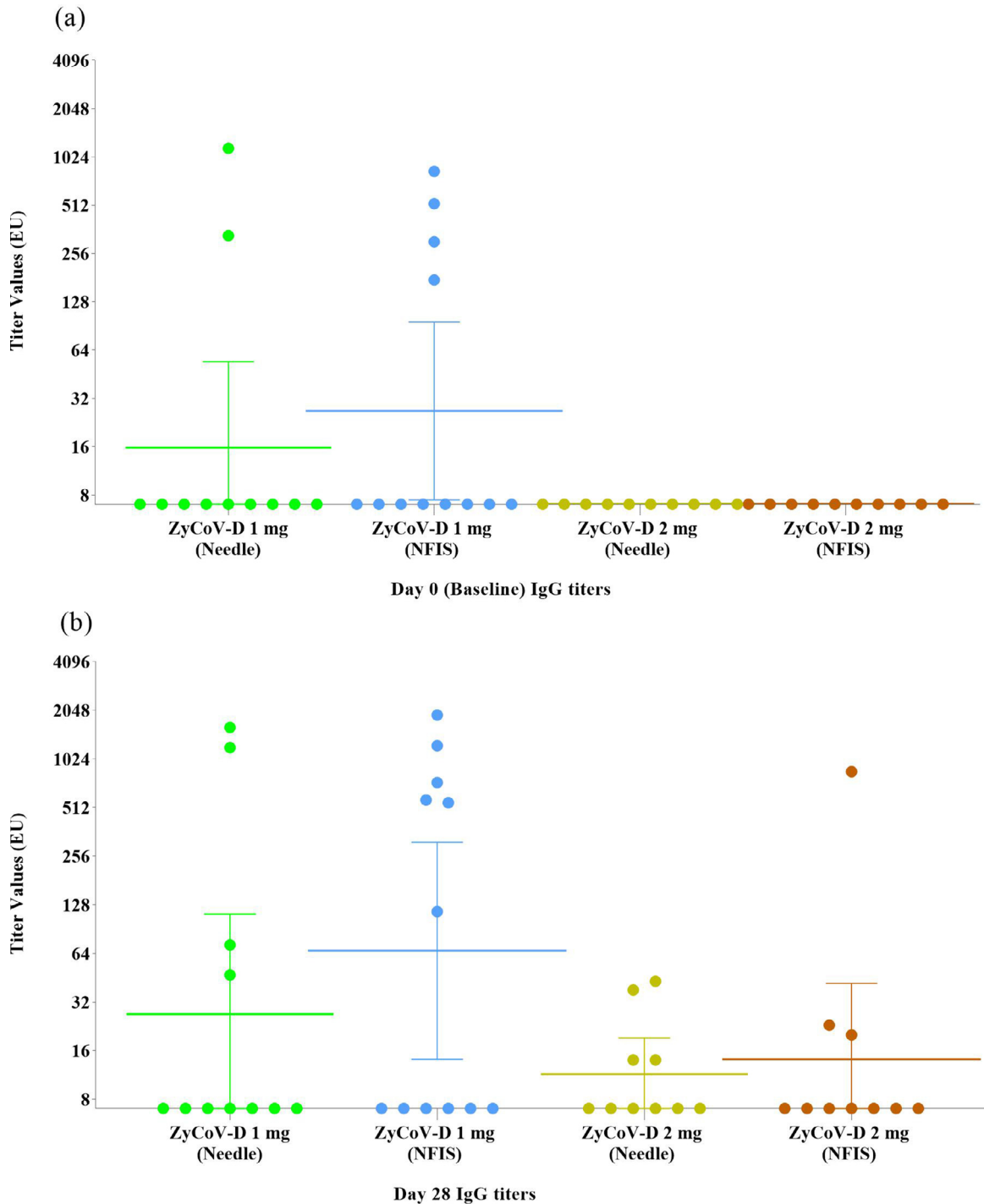
Fig. 5. (a) - 5 (f) IgG Antibody Titres with error bars, representing geometric mean titre (95%CI) at baseline (Day 0), Day 28, Day 42, Day 56, Day 70 and Day 84.

dose of vaccine and for at least 4 h post administration of the second and the third dose of vaccine. Each vaccination was followed by frequent safety follow-up with subjects till 28 days of the last dose of vaccine. ZyCoV-D vaccine was well-tolerated in 48 healthy adults in all four dose groups with no vaccine-related severe or SAEs. The safety profile of ZyCoV-D vaccine supports further development of ZyCoV-D in at-risk populations who are at more serious risk of complications from SARS-CoV-2 infection, including the elderly and subjects with comorbidities. Our findings also correlate with previous clinical evaluation of other DNA

vaccine candidates which were reported to be safe and well-tolerated in healthy subjects [17,19−21].

The majority of solicited AEs reported in this trial were after the first dose of vaccine, while the second and third dose of vaccination were found to be well-tolerated. The ZyCoV-D Phase 1 safety data further suggest that the vaccine could be a safe booster as there was no increase in frequency of side effects after the third dose compared to the first dose, an important aspect for the safety profile of SARS-CoV-2 vaccines. One attractive feature of DNA vaccines, like ZyCoV-D, is that the immunizations could be boosted without significant
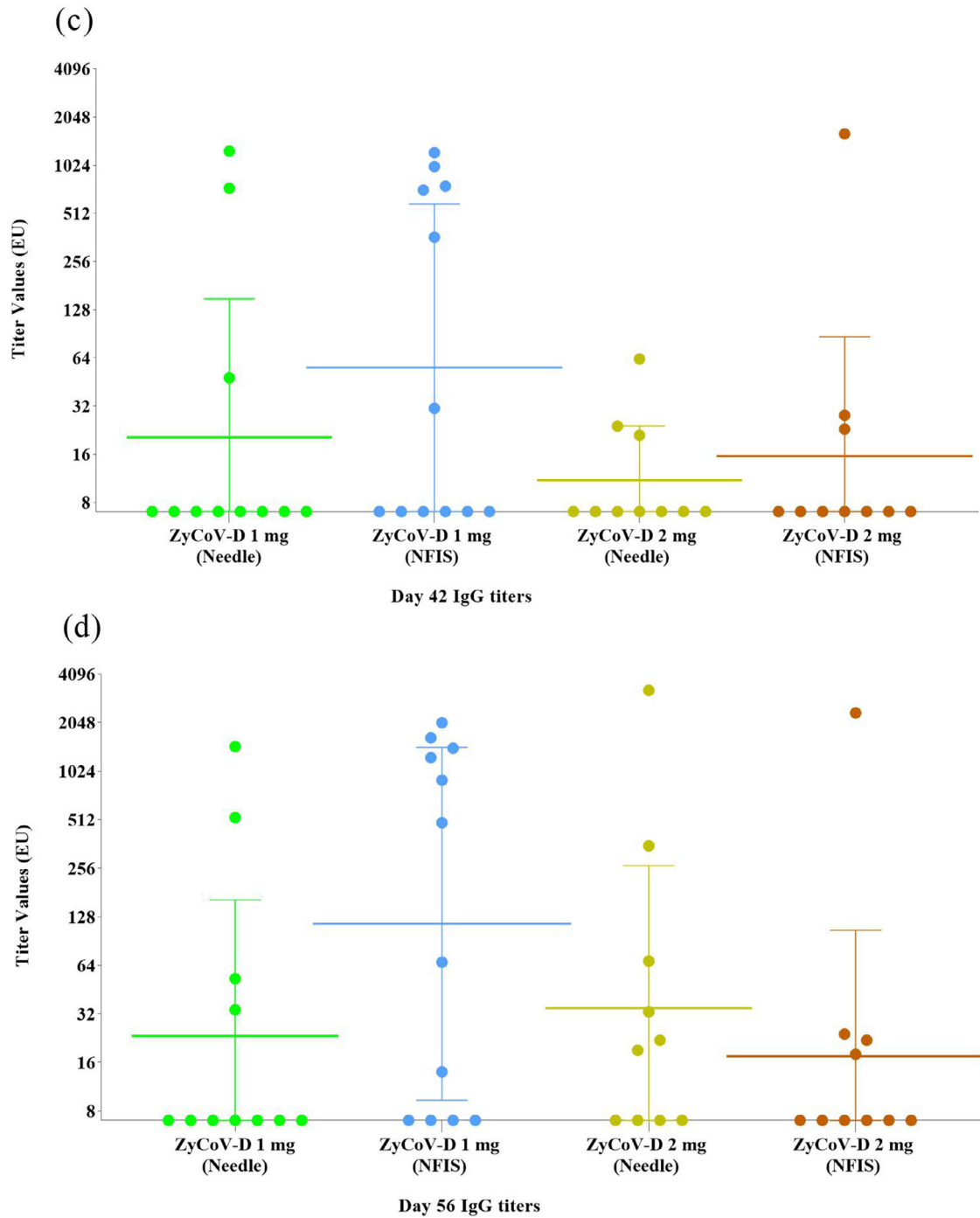
(c)

Day 42 IgG titers

(d)

Day 56 IgG titers

Fig. 5. Continued.

limitations such as dosing-incremented toxicities or anti-vector responses and additional boosting with other DNA vaccines have resulted in higher levels of cellular and humoral immune responses without increased toxicity [22].

ZyCoV-D also generated balanced humoral and cellular immune responses in participants displaying either or both antibody or *T* cell responses following three doses of vaccine. Humoral responses were lower in subjects who received 1 mg vaccine irrespective of method of administration. The exact reason for this is not known but it is likely that when the vaccine is administered at the low dose of 1 mg at single intradermal site, it may lead to inefficient transfection, in the host cells and thus lower the expression of antigen. Our data corroborates well with the Rhesus Macaques challenge study, where the vaccination of 2 mg dose with Pharmajet NFIS elicited significant SARS CoV-2 specific IgG, NAB titers and lower viral loads in animals post challenge (Data on file). Further; a Phase II study in 1000 subjects is currently ongoing which will provide better understanding of immunogenicity of ZyCoV-D vaccine in a larger sample size.

In our study, three doses of 2 mg ZyCoV-D DNA vaccine administered intradermally at two different sites via NFIS device 28 days apart have shown good humoral and cellular immune response at Day 70 onwards. Presently, correlation of protection for vaccine against SARS-CoV-2 is unknown, and the roles of the specific antibodies or T cells in building effective protection are not yet well-defined. Therefore, we are only able to demonstrate immune response induction following vaccination and not protection to SARS-CoV-2 following DNA vaccination on the basis of the vaccine-elicited immune responses in this study. A double-blind, placebo controlled Phase III
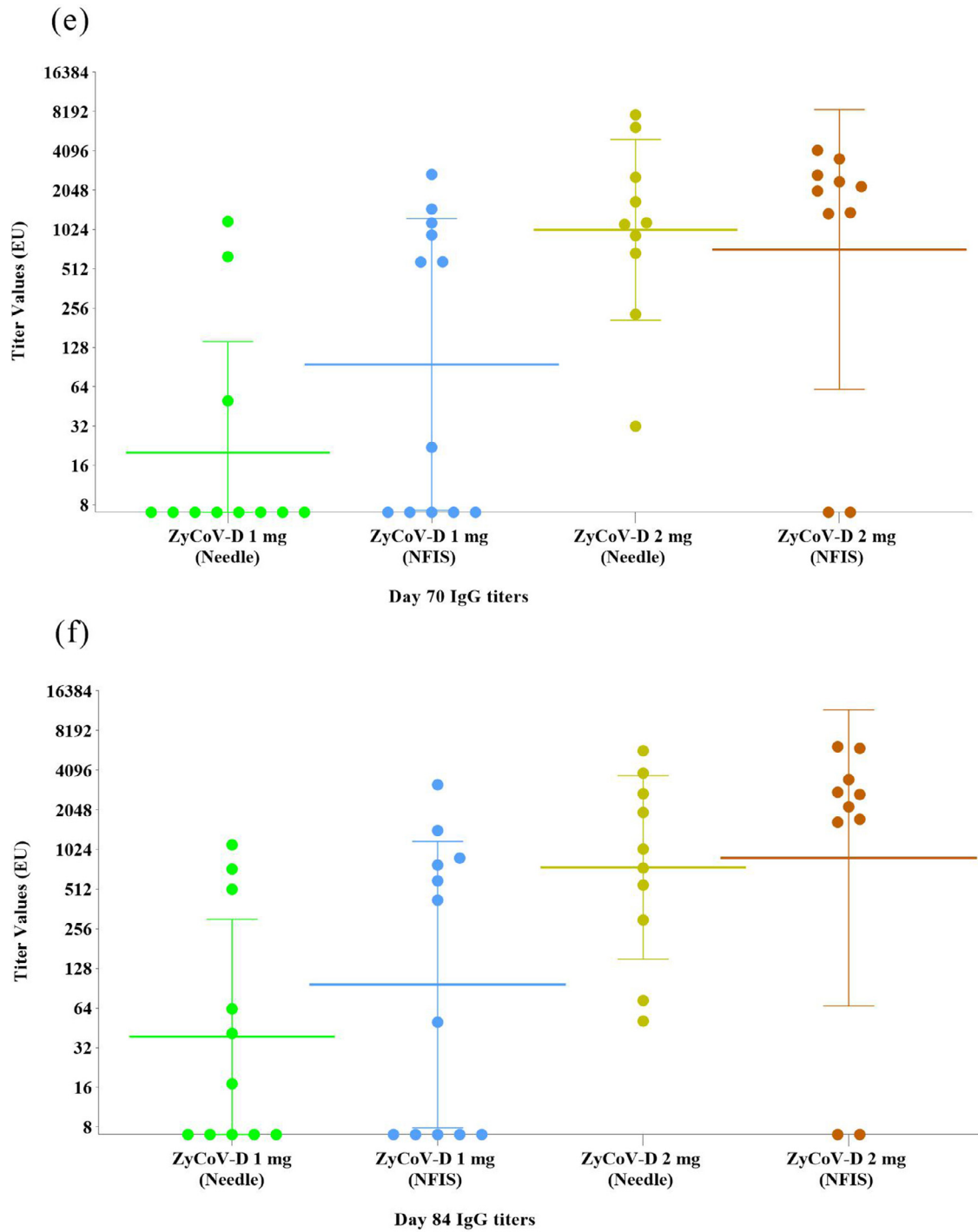
(e)

(f)

Fig. 5. Continued.

study in 28,216 subjects aged 12 years and above is also currently ongoing which will help evaluate efficacy of the ZyCoV-D 2 mg dose administered via NFIS device in protection against COVID-19 infection. The study is registered with CTRI/2021/01/030416.

Previous studies investigating SARS and Middle East Respiratory Syndrome (MERS) found that there is a temporary rise in specific antibodies which dropped rapidly in subjects after recovery, and the CD4+ and CD8+ T-cell responses played a vital role in memory response and protection against future exposure to virus [23]. A similar rapid decline of the specific antibody amounts in subjects with COVID-19 after recovery was also noted [23] suggesting that both

specific cellular and humoral immunity are potentially important for a successful COVID-19 vaccine. Here, we report immune response till 28 days after the last dose of vaccine.

ZyCoV-D vaccine also induced cellular response as measured by IFN-γ ELISPOT which was maintained till Day 84 in subjects who received vaccination 1 mg or 2 mg via NFIS device. This clearly indicates that vaccination with ZyCoV-D induces cellular response with fold rise. However, the sample size per arm is too small to reach a definitive conclusion on the levels of IFN-γ in different arms and the results should be interpreted in the context of variability of the immunological responses among individuals enrolled in the trial.

Table 4
Summary results of neutralization titers at day 28, 56 and 84.

Time Point	Statistics	ZyCoV-D 1 mg (Needle) (N = 11)	ZyCoV-D 1 mg (NFIS) (N = 12)	ZyCoV-D 2 mg (Needle) (N = 10)	ZyCoV-D 2 mg (NFIS) (N = 10)
Day-0	GMT(95% C.I)	5.98 (4.01, 8.92)	8.88 (4.52, 17.48)	5.00 (5.00, 5.00)	5.00 (5.00, 5.00)
Day-28	GMT(95% C.I)	6.79 (4.29, 10.73)	11.12 (4.91, 25.19)	5.00 (5.00, 5.00)	6.06 (3.93, 9.34)
	GMFR(95% C.I)	1.13 (0.82, 1.57)	1.25 (0.62, 2.52)	1.00 (1.00, 1.00)	1.21 (0.79, 1.87)
Day-56	GMT(95% C.I)	6.48 (3.64, 11.52)	12.99 (5.60, 30.16)	8.72 (3.08, 24.72)	6.34 (3.70, 10.87)
	GMFR(95% C.I)	1.08 (0.91, 1.29)	1.46 (0.63, 3.42)	1.74 (0.62, 4.94)	1.27 (0.74, 2.17)
Day-84	GMT(95% C.I)	8.52 (4.40, 16.49)	11.97 (5.72, 25.06)	14.13 (5.57, 35.82)	39.17 (15.43, 99.47)
	GMFR(95% C.I)	1.42 (0.83, 2.45)	1.35 (0.68, 2.65)	2.83 (1.11, 7.16)	7.83 (3.09, 19.89)

Abbreviation(s): CI = confidence interval; GMT = geometric mean titre; GMFR = geometric mean fold rise; *N* = number of subjects in respective treatment arm; *n* = number of subjects in specified category; NFIS = Needle Free Injection System.
Seroconversion defined as a positive antibody response as at least a four-fold increase in post-vaccination titer from baseline.

Table 5
Summary of Seroconversion for Neutralization Titers.

Time point	Seroconversion	ZyCoV-D 1 mg (Needle) (N = 11)	ZyCoV-D 1 mg (NFIS) (N = 12)	ZyCoV-D 2 mg (Needle) (N = 10)	ZyCoV-D 2 mg (NFIS) (N = 10)
Day-28 *n* (%)	No	10 (90.91)	10 (83.33)	10 (100.0)	9 (90.00)
	Yes	1 (9.09)	2 (16.67)	0 (0.00)	1 (10.00)
Day-56 *n* (%)	No	11 (100.0)	10 (83.33)	8 (80.00)	9 (90.00)
	Yes	0 (0.00)	2 (16.67)	2 (20.00)	1 (10.00)
Day-84 *n* (%)	No	9 (81.82)	10 (83.33)	5 (50.00)	2 (20.00)
	Yes	2 (18.18)	2 (16.67)	5 (50.00)	8 (80.00)

Abbreviation(s): *N* = number of subjects in respective treatment arm; *n* = number of subjects in specified category; NFIS = Needle Free Injection System.
Seroconversion defined as a positive antibody response as at least a four-fold increase in post-vaccination titer from baseline.

Phase II data with a higher sample size will help to understand cellular response obtained with ZyCoV-D vaccine.

This first-in-human study of ZyCoV-D DNA vaccine has some limitations. First, this open-label, non-randomized Phase 1 trial report is based on a modest sample size (48) in all vaccine arms and, therefore lacks a comparator group. Larger sample-sized randomized placebo controlled blinded trials may be needed to show the true immunogenicity difference between the dose groups. Second, this report only involves healthy Indian male subjects aged between 18 and 55 years. This is due to societal limitation, COVID 19 related lockdown and completion of recruitment with male subjects at study center. The results of this study are not generalizable to other ethnic groups and female subjects. In this regard, female subjects were part of Phase 2 and 3 studies. SARS-CoV-2 infection has more severe and fatal outcomes in older individuals. In this regard, the Phase 3 trial will evaluate individuals of higher age group. Third, only data within the first 84 days of vaccination is being reported, and this report does not include data about the durability of the vaccine-induced immunity. In previous clinical trials with similar DNA vaccines, durable immune responses up to 1 year following vaccination were reported [14,22]. Fourth, the study showed good humoral and cellular immune response at Day 70 onwards after administration of the third dose, while most other approved vaccines showed immune response after administration of the second dose.

In this study, two different vaccination strategies were used. One is injection and needle, and the second is needle-free injection. i.e. NFIS device. This technology has evolved significantly over the last 50 years and is now accepted in many routine immunization settings as a safe and effective vaccine delivery method. Disposable syringe jet injectors are now being used for the delivery of vaccines to eradicate polio, measles, mumps, rubella and influenza, and are showing promising results in vaccine clinical trials for the Zika virus and human papillomavirus. Vaccine administration using NFIS device offers some distinct advantages compared to the conventional method of vaccination using needle and syringe, like improved compliance and better coverage; no needle trash and needle stick injuries; higher immunogenic response; calibrated for specific volume with minimal vaccine wastage; auto disabling and eliminating possibility of re-use; efficient vaccine delivery; and the workflow is 25% faster than a conventional needle-syringe and is less painful [24]. Tebas et al. reported better immune response after administration of two doses with intradermal DNA vaccine followed by electroporation (EP) technique [17]. However, Pharmajet Tropis device has been used in DNA vaccine clinical trials and has been reported as a better administration technique in terms of ease of administration, reliability, and precision. Use of Tropis is also reported to be cost-effective and have better local tolerance compared with EP [25].

Our data suggests that ZyCoV-D demonstrates a good safety profile and that vaccination induces both cellular and humoral responses, supporting its further development to prevent infection and death related to COVID-19 in the global population. The safety and immunogenic profile are important parameters for vaccination for high-risk populations, such as the elderly and those living with co-morbid conditions.

Over the past decade, the vaccine industry and clinical research centers have been asked to provide urgent responses to epidemics of emerging infectious diseases, such as H1N1 influenza, Ebola virus, Zika, MERS, and now SARS-CoV-2 [26]. The risk of COVID-19 caused by SARS-CoV-2 is ongoing, making the need for effective vaccines even more urgent [27]. Previous findings suggested that those vaccines expressing full-length spike glycoprotein can induce good immune responses and protective efficacy. The full-length spike was chosen in most of the viral vectored, mRNA, or DNA COVID-19 vaccines in development [23].

There have been recent reports of emergence of new SARS-CoV-2 viral strains like B.1.1.7 in UK, B.1.351 in South Africa, P.1 in Brazil [28]. The emergence of new strains of virus has raised the doubts about efficacy of vaccines which were already approved for emergency use authorization. Currently ongoing clinical trials with ZyCoV-D vaccine will provide important insights into efficacy and safety of DNA vaccine platform. DNA vaccines are based on plug and play platform, which allows rapid development of new constructs in case mutant strains develop, and possibility of generating a new vaccine candidate in very short time, thus providing protection against mutated viral strains.

(a)

(b)

(c)

(d)
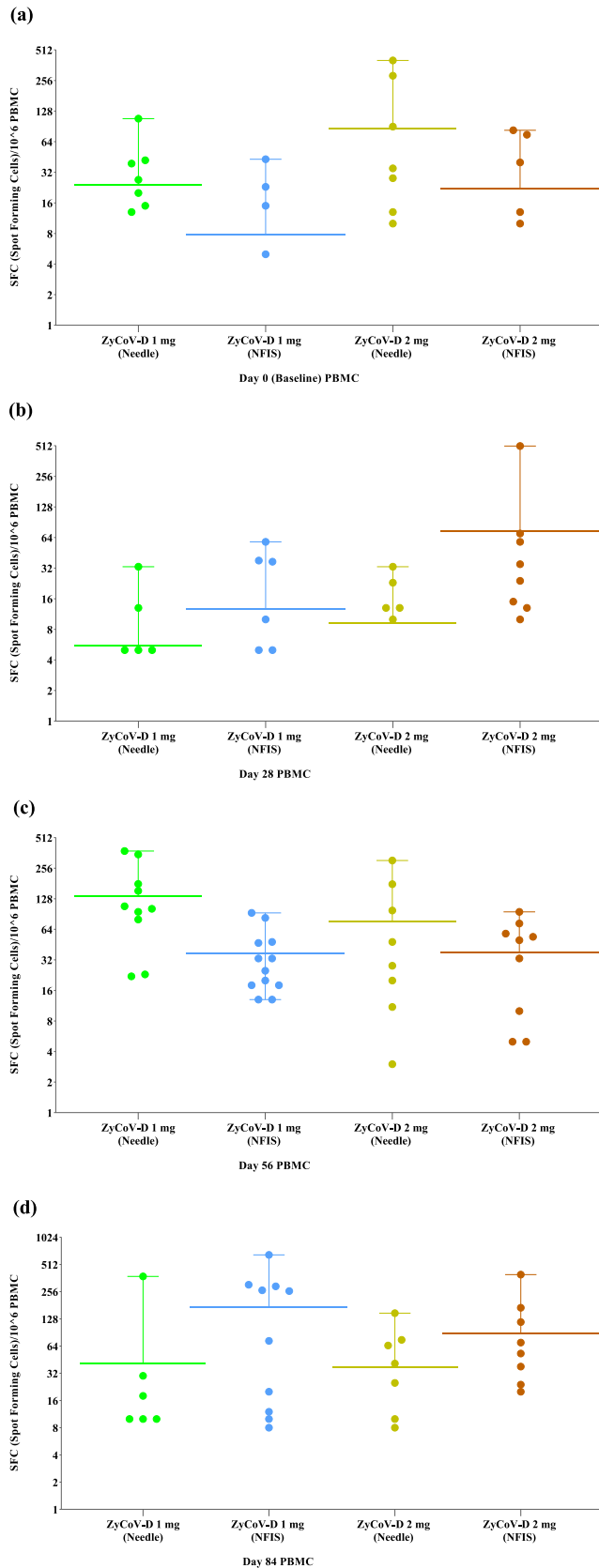
Fig. 6. (a) − (d) Cell mediated response as measured by SPC (spore forming cells) in millions PBMC with error bars, representing mean (range) at baseline (Day 0), Day 28, Day 56, and Day 84.

Declaration of Competing Interest

All authors declared no competing interests. TM, KK, HP, SS, BS, JP, RM, JS, KM, AD, HC, CR, HPR, PK and AN are employee of Cadila Healthcare Limited, Ahmedabad, India. DP is an employee of Zydus Discovery DMCC, Dubai, United Arab Emirates.

Contributors

KK, JS, RM and DP were involved in conceptualization of the study. TM and HP were the study investigators. TM and JP were involved in data interpretation, manuscript writing, and manuscript review. SS was involved in statistical analysis, designing, programming and generation of Tables, Listing, Figures and aided in interpretation of results. BS was a pharmacist for this study. KM was involved in conceptualizing, designing, developing the vaccine candidate and guiding on data analysis, AD was involved in designing, developing vaccine candidate, perform data analysis for ELISPOT and Luminex assay. HC and CRTM were involved in development of analytical procedures for testing of the vaccine and data analysis for ELISA, neutralization. HPRP was involved in developed process for vaccine production and manufacture Phase-1 vaccine batches, AN was the responsible for quality assurance and regulatory support. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. All authors approved the final version of the manuscript for submission.

Data sharing statement

Deidentified data are in the process of being deposited on the Data Repository for the Cadila Healthcare Limited, and the corresponding author can be contacted for data access.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2021.101020.

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