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Safety and immunogenicity of a prefusion non-stabilized spike protein mRNA COVID-19 vaccine: a phase I trial

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Effective mRNA SARS-CoV-2 vaccines are available but need to be stored in freezers, limiting their use to countries that have appropriate storage capacity. ChulaCov19 is a prefusion non-stabilized SARS-CoV-2 spike-protein-encoding, nucleoside-modified mRNA, lipid nanoparticle encapsulated vaccine that we report to be stable when stored at 2–8 °C for up to 3 months. Here we report safety and immunogenicity data from a phase I open-label, dose escalation, first-in-human trial of the ChulaCov19 vaccine (NCT04566276). Seventy-two eligible volunteers, 36 of whom were aged 18–55 (adults) and 36 aged 56–75 (elderly), were enroled. Two doses of vaccine were administered 21 d apart at 10, 25 or 50 μg per dose (12 per group). The primary outcome was safety and the secondary outcome was immunogenicity. All three dosages of ChulaCov19 were well tolerated and elicited robust dose-dependent and age-dependent B- and T-cell responses. Transient mild/moderate injection site pain, fever, chills, fatigue and headache were more common after the second dose. Four weeks after the second dose, in the adult cohort, MicroVNT-50 geometric mean titre against wild-type SARS-CoV-2 was 848 (95% CI, 483–1,489), 736 (459–1,183) and 1,140 (854–1,522) IU ml^{-1} at 10, 25 and 50 μg doses, respectively, versus 285 (196–413) IU ml^{-1} for human convalescent sera. All dose levels elicited 100% seroconversion, with geometric mean titre ratios 4–8-fold higher than for human convalescent sera ($P < 0.01$), and high IFNγ spot-forming cells per million peripheral blood mononuclear cells. The 50 μg dose induced better cross-neutralization against Alpha, Beta, Gamma and Delta variants than lower doses. ChulaCov19 at 50 μg is well tolerated and elicited higher neutralizing antibodies than human convalescent sera, with strong T-cell responses. These antibodies cross-neutralized four variants of concern. ChulaCov19 has proceeded to phase 2 clinical trials. We conclude that the mRNA vaccine expressing a prefusion non-stabilized spike protein is safe and highly immunogenic.

More than 10 billion doses of COVID-19 vaccines have been administered worldwide but only 10% of those living in low-income countries have received at least one dose (8 February 2022)[1]. Among approved vaccines, mRNA vaccines have the highest efficacy[2]. Building capacity to develop and manufacture mRNA vaccines in low- to middle-income countries (LMIC) is important for the current and future pandemics.

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The ChulaCov19 mRNA vaccine (ChulaCov19) is a lipid nanoparticle (LNP)-encapsulated nucleoside-modified mRNA encoding a non-stabilized SARS-Cov-2 spike protein. This spike protein consists of an extracellular domain with no transmembrane and cytoplasmic domains; therefore, it will be a secreted spike protein. Two major differences between ChulaCov19 and the Pfizer/BioNTech and Moderna vaccines are that ChulaCov19 is not prefusion stabilized and it is encapsulated in a different LNP formulation.

One of the major constraints for LMICs in developing and manufacturing mRNA vaccines is the patent rights protection and licensing issue. In most instances, companies that own or otherwise control COVID-19 vaccines approved for clinical use have retained licensing and control over vaccine production. To overcome this obstacle, we adopted a different payload strategy, encoding a prefusion non-stabilized form of the spike protein with an alternate lipid nanoparticle composition.

Besides this vaccine being more economically feasible for LMICs to implement, we also tested its stability at 2–8 °C. ChulaCov19 was designed in Thailand and manufactured in North America for early clinical trials, in parallel with large-scale production capacity development in Thailand. In rodent and macaques, ChulaCov19 elicited strong B- and T-cell responses[3].

Results

Enrolment and follow-up

Between 28 May 2021 and 2 July 2021, 132 individuals were screened (36 were enroled in each age cohort, with 12 per dose group in each age cohort) (Extended Data Figs. 1 and 2). All but one participant completed both vaccinations. The participant declining a second vaccination was from the adult 25 µg group and experienced moderate myalgia after the first vaccination, which resolved within 3 d and was accompanied by moderate creatinine phosphokinase elevation but normal cardiac enzymes, possibly related to strenuous physical labour. This participant was excluded from the per protocol immunogenicity analysis, as were two others: one participant from the adult 10 µg group who was diagnosed with asymptomatic COVID-19 on the sixth day after the second vaccination and one participant from the adult 50 µg group who had confirmed positive baseline anti-receptor binding domain (RBD) binding antibody test but was negative for SARS-CoV-2 molecular testing, indicating asymptomatic COVID-19 before enrolment. All participants contributed to the safety analysis.

Demographic and baseline clinical characteristics

Characteristics of the study participants are listed in Table 1. Mean ages(s.d.) in the adult and elderly cohorts were 35.8 (9.1) and 65.1 (5.4) yr, respectively; body mass indices were 23 (2.8) and 23.3 (3.3), and proportions of females were 67% and 53%, respectively.

Table 1 | Demographic characteristics of the participants by dose age groups

Characteristic	10 µg group	25 µg group	50 µg group	Overall
Adults (aged 18–55 yr)	*N*=12	*N*=12	*N*=12	*N*=36
Sex-number (%)				
Female	9 (75.0)	7 (58.3)	8 (66.7)	24 (66.7)
Age-Mean (s.d.)	35.9 (5.9)	34.1 (9.0)	37.3 (11.8)	35.8 (9.1)
Min–max	21–44	26–53	21–53	21–53
Body mass index ($kg\,m^{-2}$), Mean (s.d.)	23.9(3.3)	22.1(2.6)	22.9 (2.5)	23.0 (2.8)
Elderly (aged 56–75 yr)	*N*=12	*N*=12	*N*=12	*N*=36
Sex-number (%)				
Female	7 (58.3)	7 (58.3)	5 (41.7)	19 (52.8)
Age-Mean (s.d.)	67.4 (3.2)	67.8 (3.2)	60 (5.5)	65.1 (5.4)
Min–max	64–73	65–74	56–71	56–74
Body mass index ($kg\,m^{-2}$), Mean (s.d.)	24.1 (4.2)	22.7 (3.1)	23.2 (2.5)	23.3 (3.3)

Safety and tolerability of ChulaCov19

The most common local reaction was injection site pain. Its incidence was dose-dependent, more common in adults than the elderly, and after Dose 2. All local reactions were mild in the elderly; some moderate events occurred in adults. All participants recovered after 2.79 ± 1.7 and 1.91 ± 0.9 d on average in adults and the elderly after Dose 2, respectively. A single event of severe erythema in the adult 10 µg group after Dose 2 resolved within 6 d (Fig. 1).

The three most common systemic reactions were fever, headache and fatigue. Systemic reactions were more common at the 25 µg and 50 µg doses, after Dose 2, and in adults. Most reactions were mild to moderate and transient, with mean duration of 1.97 ± 1.2 d in adults and 1.39 ± 0.5 d in the elderly after Dose 2 (Fig. 1).

Immunogenicity of ChulaCov19

SARS-CoV-2 RBD antibody responses. In the adult cohort 4 weeks after the second vaccination (day 50), the geometric mean titre (GMT) of anti-RBD antibody was significantly higher than for human convalescent sera 4 weeks after COVID-19 diagnosis (Fig. 2a), with geometric mean ratios (GMR) of 3.78 (95%CI 1.64–8.68), 3.23 (1.41–7.42) and 7.74 (3.37–17.77), respectively ($P < 0.01$) (Supplementary Table 10). In the elderly cohort, anti-RBD GMTs were also dose-dependent but lower than observed in the adult cohort (Fig. 2a and Supplementary Table 6).

In the adult cohort at day 50, ChulaCov19 at 50 µg elicited the highest anti-spike GMT at 24,493.9 binding antibody units ml^{-1} ($P < 0.01$). In the elderly cohort, the anti-spike GMT was lower than that of the adults at all three doses, while anti-spike GMT for the 50 µg dose was significantly higher than for the 10 µg dose ($P < 0.001$, Fig. 2b). GM of % RBD-ACE2 binding inhibition showed a dose-dependent pattern in both age cohorts at day 29 but not at day 50. All doses at day 50 in both age cohorts elicited GM > 90% inhibition. Inhibition (%) of the human convalescent serum (HCS) and Pfizer/BNT vaccinee's (day 29) panels were 76% and 93%, respectively (Supplementary Fig. 1).

SARS-CoV-2-specific live-virus micro-neutralization tests (MicroVNT-50). Seroconversion rate on MicroVNT-50 in all dose groups reached 100% by day 50. GMT of the responses against wild-type (WT) virus were both dose- and age-dependent, and markedly increased from day 29 to day 50 for the 10, 25 and 50 µg doses (Fig. 2c). In the adult cohort at day 50, ChulaCov19 at 10, 25 and 50 µg doses induced significantly higher MicroVNT-50 GMT than HCS at a ratio of 2.98 (95%CI 1.63–5.44), 2.59 (1.42–4.72) and 4.01 (2.2–7.31), respectively ($P < 0.01$), whereas in the elderly cohort the values were 0.48 (0.25–0.92), 1.26 (0.67–2.36) and 2.09 (1.12–3.91), respectively (Supplementary Table 7). ChulaCov19 at the 50 µg dose induced significantly higher MicroVNT-50 GMT against WT and 3 variants compared with the 10 µg dose in both age cohorts, while elicited MicroVNT-50 GMTs were lower for all doses in the elderly cohort compared with the adult cohort (Fig. 3a and Supplementary Table 8).

Pseudovirus neutralization tests (PsVNT-50). In the adult cohort, PsVNT-50 GMTs (95% CI) of ChulaCov19 against WT at day 50 for 10, 25 and 50 µg and for the HCS panel were 885.3 (95%CI, 438.2–1,788.7), 902.9 (445.5–1,829.7), 1,273.1 (874.2–1,854) and 471.2 (290.5–764.5), respectively (Fig. 2d). The GMT ratio of 50 µg dose/HCS was 2.7 ($P = 0.01$). At day 29, ChulaCov19 at 50 µg dose induced significantly

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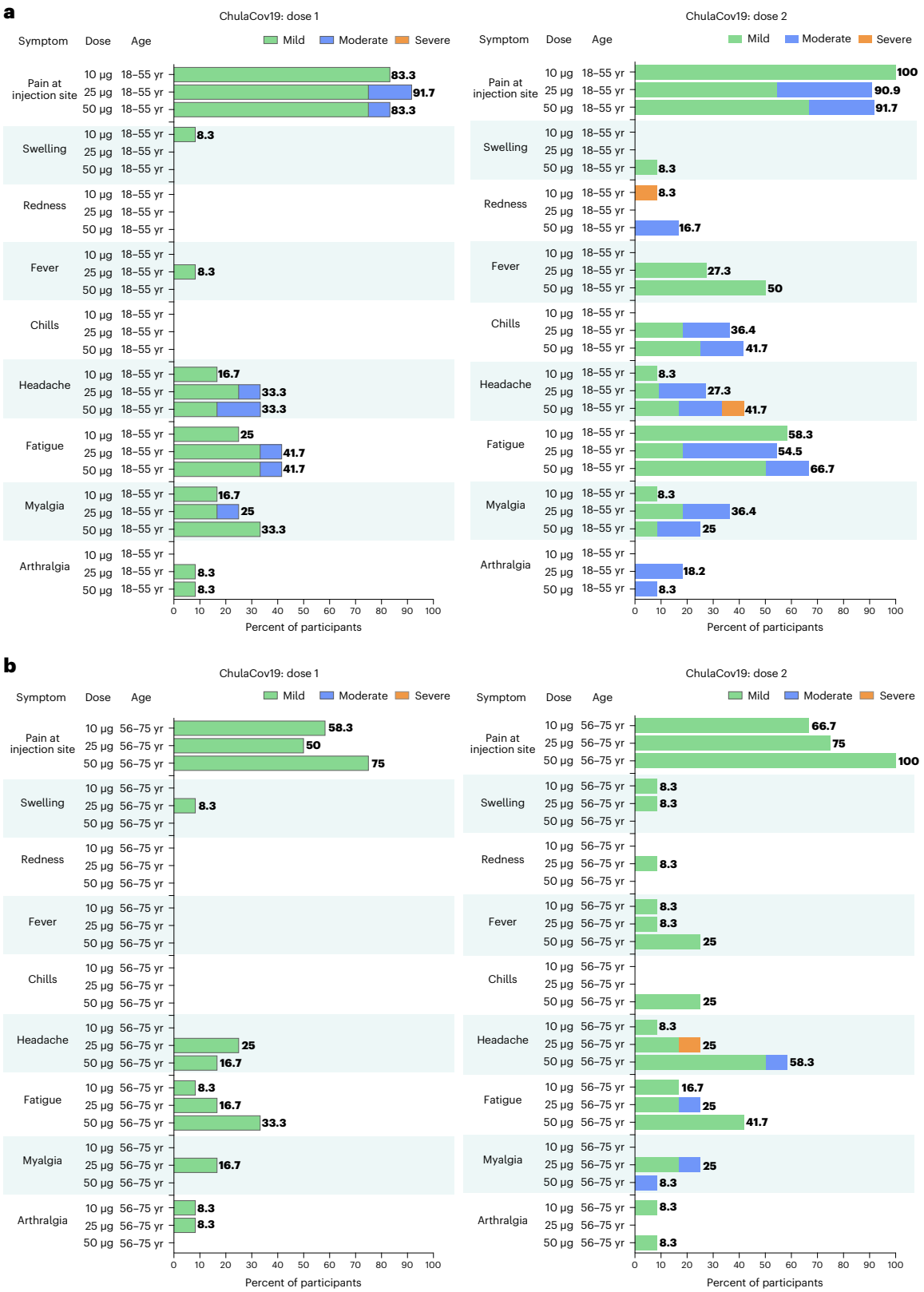

Fig. 1 | Local and systemic adverse events by dose group and age cohort. a,**b**, Severity of solicited local and systemic reactions in the adult cohort (18–55 yr old) (**a**) and the elderly cohort (56–75 yr old) (**b**). Data on local and systemic reactions were collected using paper diaries for 7 d after each vaccination. Injection-site (local) reactions were: pain at injection site (mild: does not interfere with activity; moderate: interferes with activity; severe: prevents daily activity; and grade 4: emergency room visit or hospitalization), redness and swelling (mild: 2.5–5.0 cm in diameter; moderate: 5.1–10.0 cm in diameter; severe: >10.0 cm in diameter; and grade 4: necrosis or exfoliative dermatitis). Solicited systemic events were: fever (mild: 38.0–38.4 °C; moderate: 38.5–38.9 °C; severe: 39.0–40.0 °C; and grade 4: >40.0 °C), chills, headache, fatigue, myalgia and arthralgia (all were graded as mild and did not interfere with the daily activities of the participants), moderate (interferes with daily activities), severe (prevents daily activities) or grade 4 (led to an emergency department visit or hospitalization). The numbers above the bars show the overall percentage of the participants in each group who reported the specified systemic event.

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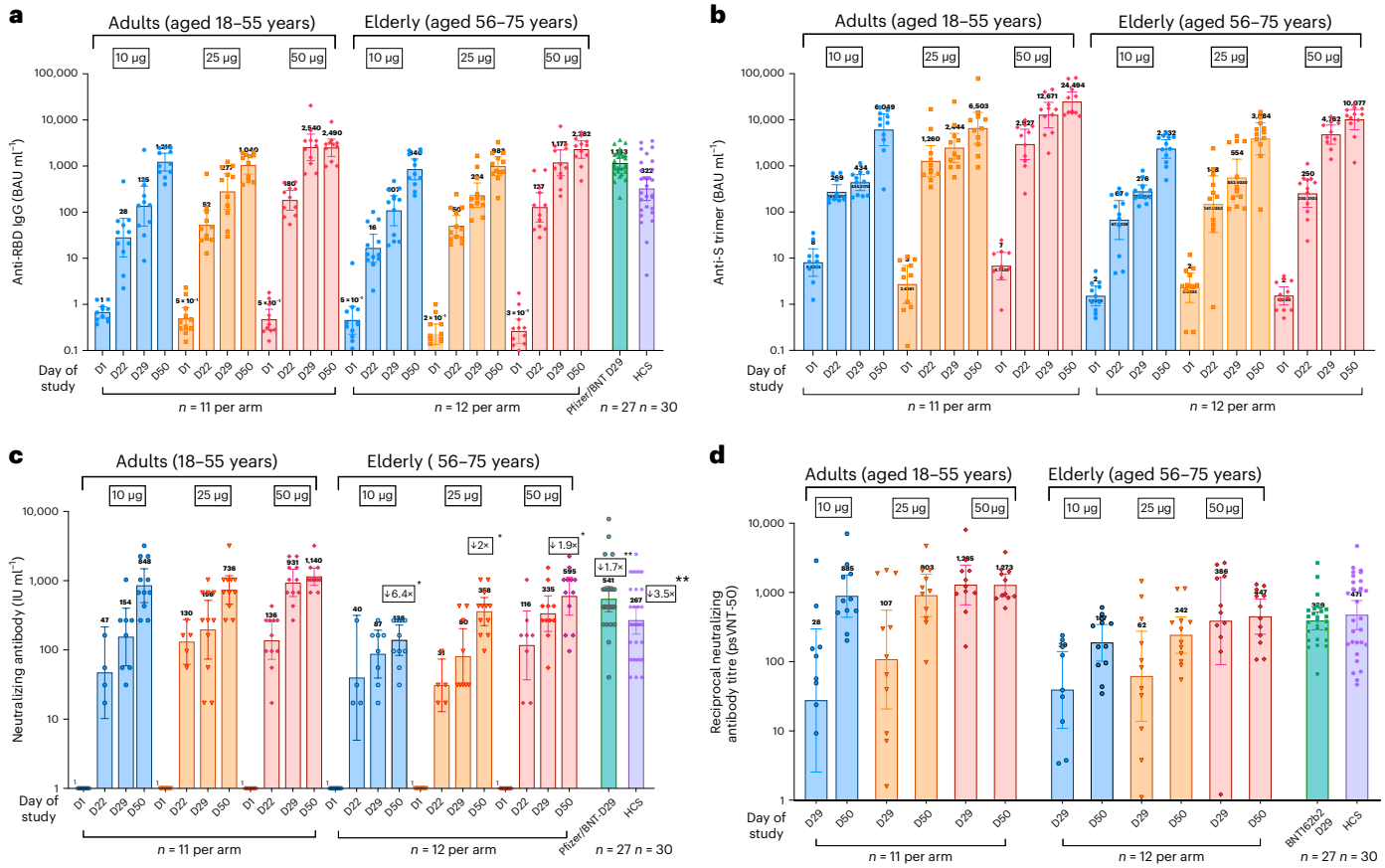

Fig. 2 | Immunogenicity assay responses to ChulaCov. a, The GMTs of anti-RBD antibody measured by ELISA. **b**, The GMTs of IgG antibody against S trimer protein of SARS-CoV-2 measured by ELISA. **c**, The GMTs of neutralizing antibody against wild-type SARS-CoV-2 (Micro-VNT50). *Fold-difference as compared to the adults' GMT, **Comparison of GMTs of 50 μg dose in adults at day 29. **d**, The GMTs of pseudovirus neutralizing antibody against wild-type SARS-CoV-2 (PsVNT-50). The right side of **a**, **c** and **d** indicate two reference values from 27 Pfizer/BioNTech mRNA Malaysian vaccinees. The serum samples were collected on day 29 after the first dose. Thirty HCS were collected at 4 weeks after confirmed diagnosis. Error bars indicate 95% confidence intervals. The numbers above the bars show the GMT of each group.

higher GMT than the Pfizer/BNT vaccinated panel against Alpha, Beta and Gamma variants at a ratio of 4.47, 11.94 and 4.23 ($P = 0.01$), respectively (Fig. 3b and Supplementary Table 9). PsVNT-50 test results for a new variant, Omicron, showed that two doses of ChulaCov19 at 50 mg elicited a substantial decline in GMT against the Omicron variant compared with WT (Supplementary Fig. 2).

SARS-Cov-2 spike-specific T-cell responses. At day 29, all participants in the adult and elderly cohorts at 10, 25 and 50 μg showed strong spike-specific T-cell responses measured by IFNγ-ELISpot tests. The responses were lower in the elderly at 10 and 25 μg than in adults (Fig. 4a and Supplementary Table 11). In adults, both spike-specific IFNγ+CD4+ and IL2+CD4+ T-cell percentages were notably higher in the 25 and 50 μg dose recipients compared with the 10 μg dose (Supplementary Table 12). ChulaCov19 elicited spike-specific Th1-dominated responses (Fig. 4b).

Vaccine stability. We assessed ChulaCov19 appearance, pH, osmolality and lipid content under varying storage conditions. The key parameters related to vaccine stability are particle size, mRNA integrity and mRNA encapsulation. We detected no substantial change in these parameters at −75 °C for up to 9 months, at −20 °C for up to 6 months, or at 2–8 °C for up to 3 months. However, there was a small increase in particle size (72, 76 vs 78 nm, respectively; nevertheless, all remained within the acceptance criteria of 50–120 nm) when vaccine was stored at 2–8 °C at 6 months. Moreover, the mRNA integrity further decreased after 6 months at 2–8 °C, to 54% as compared to at −20 °C. Thus, all parameters remained in line with the vaccine specification at 6 months for all storage conditions and there were no substantial changes for any parameter tested at −75 °C and −20 °C. There were no remarkable changes in any parameter after 3 months storage at 2–8 °C and small changes after 6 months, but all the results remained within specification and where specifications are not set, the changes were minimal and would not be expected to have any impact on the functionality of the LNP (although note that this was not tested). These conclusions will be verified by future stability studies to be performed in future clinical development (Supplementary Table 15).

Discussion

To increase access to effective COVID-19 mRNA vaccines in LMICs, and to be prepared for this and any future viral pandemics, complete mRNA-LNP vaccine development and manufacturing value chains need to be established in these countries. Unlike currently approved mRNA vaccines, such as Pfizer/BNT and Moderna[4], ChulaCov19 is a wild-type non-stabilized spike-protein-encoded mRNA vaccine encapsulated with a different LNP that is thermostable at 2–8 °C for at least 3 months. In a phase 1 trial, three dosages of ChulaCov19 vaccine were well tolerated, with no serious adverse effects observed in either age-group. Injection site pain was the most common adverse effect, while fever, chills, headache and myalgia were reported to be dose-dependent and more frequent after the second dose. Adverse effects were both less frequent and milder among the elderly participants. Moderate to severe

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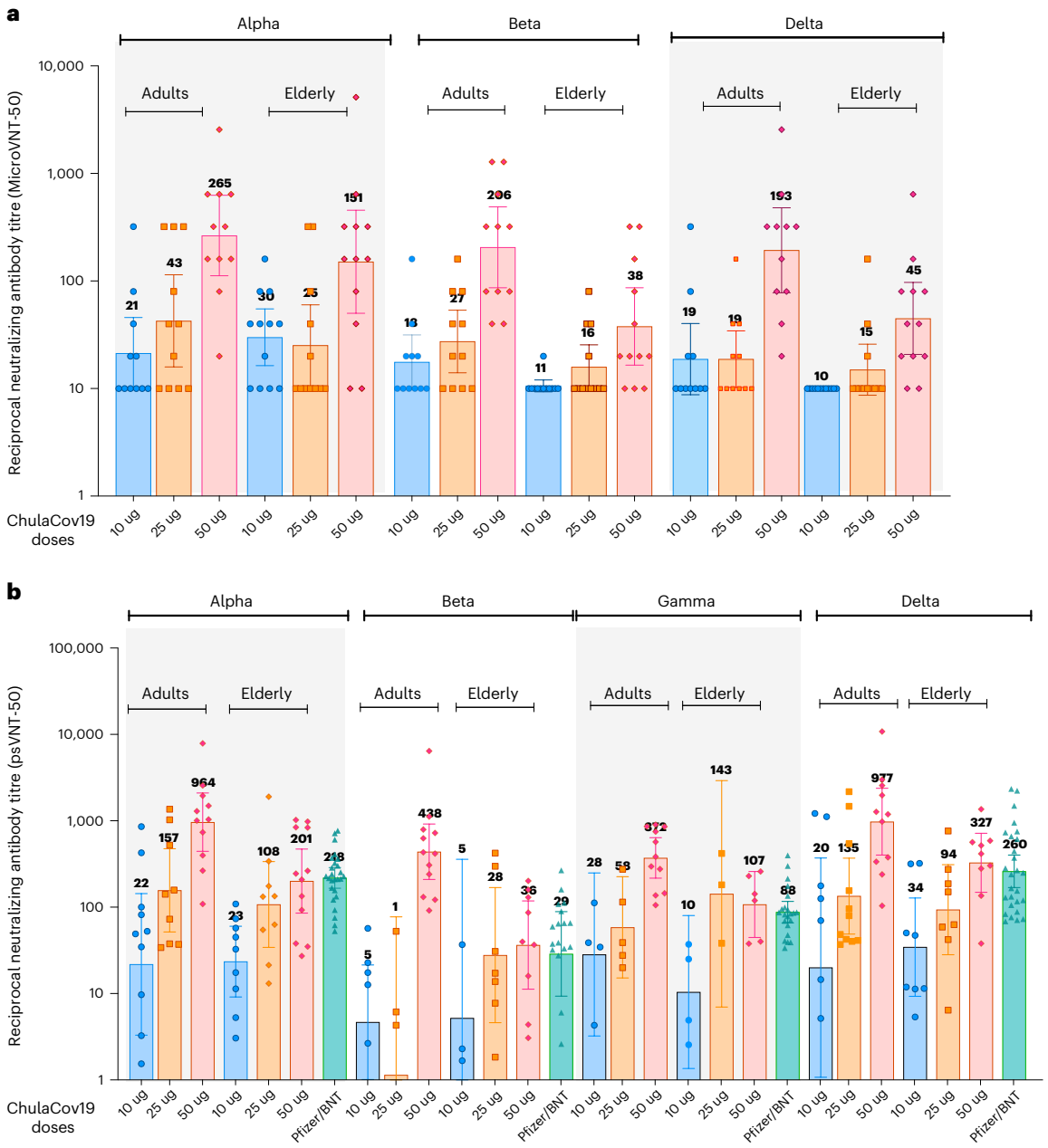

Fig. 3 | SARS-CoV-2 neutralization responses against VOCs. The serum samples were collected on day 29 after the first dose from participants in each cohort (adult $n = 11$ per dose, elderly $n = 12$ per dose). The numbers above the bars show the GMT in the group. Error bars indicate 95% confidence intervals. **a**, The GMTs of neutralizing antibody against the SARS-CoV-2 VOCs Alpha, Beta and Delta. **b**, The GMTs of pseudovirus neutralizing antibody against SARS-CoV-2 VOCs Alpha, Beta, Gamma and Delta. The right side of **b** indicates the reference values from 27 Pfizer/BioNTech mRNA Malaysian vaccinees. The serum samples were collected on day 29 after the first dose.

events were rare overall and all adverse effects resolved on average within 2.5 d.

The results indicate that this LNP-encapsulated non-di-Proline-stabilized spike-protein mRNA vaccine is strongly immunogenic. ChulaCov19 at 50 µg dose induced high SARS-CoV-2-binding and neutralizing antibodies 1 week after dose 2 ($P < 0.01$), with a microVNT-50 GMT 6-fold higher than those of HCS. At 4 weeks after dose 2, there was a further rise in all tested antibodies and all 3 doses elicited microVNT-50 GMTs higher than those of HCS, with a GMT ratio that is 4–8-fold ($P < 0.01$). The 50 µg dose induced higher microVNT-50 and psVNT-50 GMTs against tested variants than the lower doses ($P < 0.01$). In the adult cohort, at 1 week after dose 2, the 50 µg dose elicited psVNT-50 GMTs against Alpha, Beta and Gamma variants at ratios that were 4.5, 11.9 and 4.2, respectively, which are higher than those of the Pfizer/BNT vaccine ($P < 0.01$). In terms of the Omicron variant, as reported in approved Covid-19 mRNA vaccines, two doses of the ChulaCov19 vaccine may not be effective and a third dose is required[5]. Developing pan-SARS-Cov-2 or pan-Coronavirus vaccine against future pandemics is warranted[6].

All doses of ChulaCov19 generated strong T-cell responses and the higher doses (25 and 50 µg) elicited higher % of spike-specific IL2+CD4+ T cells and % of IFNγ+CD4+ T cells than the 10 µg dose ($P < 0.01$ and $P < 0.05$, respectively). At all doses, ChulaCov19 elicited predominantly SARS-CoV-2-specific Th1-type responses. On the basis of these results,

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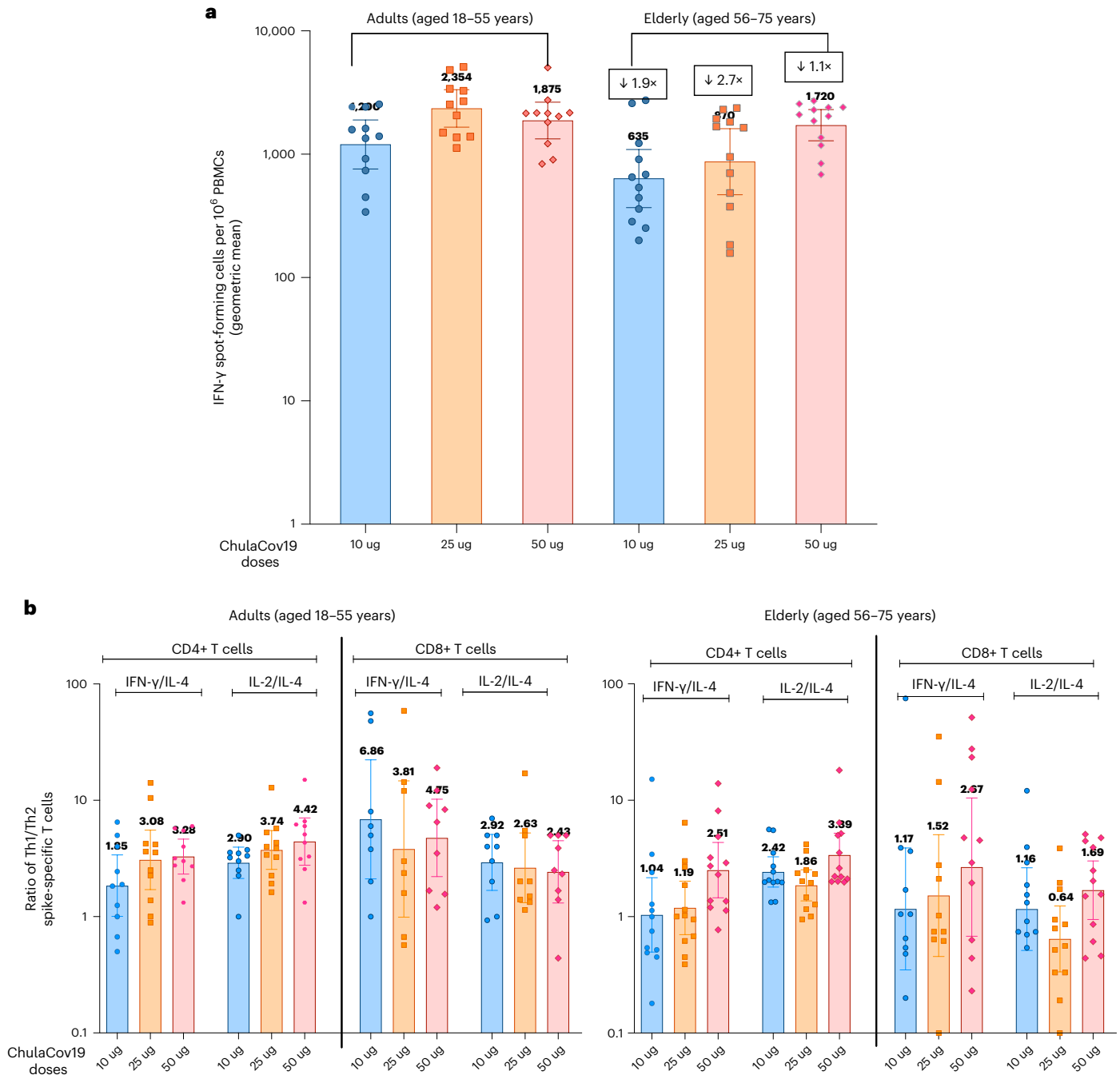

Fig. 4 | Magnitude of ChulaCov-induced CD4+ and CD8+ T-cell responses. PBMCs were obtained on day 29 after the first dose from participants in each cohort (adult $n = 11$ per dose, elderly $n = 12$ per dose). The numbers above the bars show the GMT in the group. Error bars indicate 95% confidence intervals. **a**, SARS-Cov-2 wild-type spike-specific IFN-γ ELISPOT T cells. *Comparison of the adults' GMTs shown as fold-difference. **b**, SARS-CoV-2 spike-protein-specific CD4+ and CD8+ T-cell responses, and Th1/Th2 polarization responses quantified by intracellular cytokine staining.

the Data Safety Monitoring Board (DSMB) has recommended that ChulaCov19 be further advanced to a phase 2 randomized-controlled trial with the 50 µg dose.

This vaccine did not use prefusion stabilization of spike with two prolines (K986P/V987P), which many approved COVID-19 vaccine platforms use[7]. The phase I data presented here show that ChulaCov19 elicited robust humoral and T-cell responses, which were often greater than those of the BioNTech/Pfizer vaccine that contains the di-proline modification. There are other differences in the BioNTech/Pfizer modified mRNA vaccine that could account for the greater potency of ChulaCov19, including different untranslated regions, coding sequence optimization, poly(A) tail and the LNP formulation used. Our data support the idea that the spike does not need to be prefusion stabilized to induce a potent and protective response, unlike respiratory syncytial virus[8].

Previous studies suggest that a GMT ratio of neutralizing or binding antibody in vaccinees against wild-type virus to the level in convalescent patients of >1 is associated with >70% vaccine efficacy rate[2]. In addition, higher binding and neutralizing antibody levels at 4 weeks after the second dose were found to correlate with symptomatic

infection risk reduction in the AZD1222 trial[9] and COVE study[10]. ChulaCov19 induced strong binding and neutralizing antibody responses 4 weeks after the second dose, and the ratio of neutralizing antibody GMT in ChulaCov19 vaccinees vs convalescent sera of 4 weeks after diagnosis of approximately 4–8-fold suggests that this vaccine candidate has a potentially significant vaccine efficacy. A consensus on correlates of protection may be a challenge[11] and recently the International Coalition of Medicines Regulatory Authorities has accepted well-designed immunobridging studies for authorizing COVID-19 vaccines[12]. However, due to escape of the Omicron variant from current approved vaccines, the efficacy of all current vaccines is reduced and a new variant-specific analysis is therefore required[13–15]. One of the limitations of currently approved mRNA COVID-19 vaccines is that recent data have shown that the neutralizing antibody (NAb) against WT and Omicron induced by 2 doses of approved COVID-19 vaccines wane over time. In addition, due to a greater vaccine escape of Omicron variants, the cross-neutralizing titre against the Omicron variant is several folds lower than against WT, therefore the decline rate is much faster[15]. Administering a third dose of COVID-19 mRNA vaccine as a booster has enabled a substantial increase in GMT against WT, Delta and Omicron variants[16]. In our study, administration of 2 doses of ChulaCov19 vaccine induced neutralizing antibodies with less potency for the Omicron variant, although only mild-to-moderate reductions in efficacy against other variants of concern (VOCs) were observed (Fig. 3). It is therefore likely that dual vaccinations with ChulaCov19 vaccine may need a third booster dose, ideally with a second-generation vaccine.

Accessibility to effective COVID-19 vaccines, particularly mRNA vaccines, remains very limited in many LMICs[17], and the main goal of ChulaCov19 development is to be part of the solution to this problem. There are several challenges to overcome: clinical development to reach emergency use authorization (EUA), establishing large-scale manufacturing capacity, negotiation of expanding LNP-licensing territory, and the cost and long lead-time of raw materials. BioNet Asia, Thailand has already established manufacturing capacity for both mRNA production and encapsulation, and a first clinical lot has been released for further clinical development and EUA approval.

The limitations of this study include the small sample size and dose-finding design. The exploratory comparative immunogenicity analyses with convalescent sera or Pfizer/BNT vaccinees' sera are not head-to-head comparisons and are not free from bias. Convalescent sera were collected during the Delta wave in Thailand, and it is possible that human convalescent sera antibody responses are stronger against Delta than against WT. To minimize bias, the convalescent and Pfizer/BNT vaccinees' serum samples were tested at the same laboratories together with the ChulaCov19 vaccinated samples. In addition, an randomized controlled trial phase 2 study has begun and a larger-scale immune-bridging, non-inferiority phase 3 study is planned.

In summary, we provide presumably the first evidence in humans that an mRNA vaccine expressing a prefusion non-stabilized spike protein is safe and highly immunogenic, similar to an approved mRNA vaccine, Pfizer/BNT, expressing a prefusion spike protein stabilized by the addition of a di-proline mutation. ChulaCov19 mRNA vaccine is well tolerated, elicited strong SARS-CoV-2-specific B- and T-cell immunogenicity, and is currently under phase 2 and later clinical development.

Methods

Trial design

This phase 1, open-label, dose-escalation study to evaluate safety and immunogenicity of ChulaCov19 vaccine enroled healthy participants aged 18–55 (adults, $n = 36$), followed by a cohort aged 56–75 (elderly, $n = 36$). The inclusion and exclusion criteria are shown in the Supplementary Table 1. All participants meeting eligibility criteria were randomized sequentially in a 1:1:1 allocation ratio into each dose schedule. Both age cohorts received ChulaCov19 at 10, 25 or 50 μg per dose, with 12 participants per dose group for each age cohort, in a sentinel dose-escalating manner.

The trial and the Investigational New Drug application were approved by the ethics committee of the Faculty of Medicine, Chulalongkorn University, Bangkok, and Thailand's Food and Drug Administration, respectively. All participants provided written informed consent. The trial was conducted at the Chula Clinical Research Center and King Chulalongkorn Memorial Hospital, Bangkok.

Trial vaccine

ChulaCov19 mRNA was manufactured at Trilink Biotechnologies (San Diego, California), and the mRNA-LNP vaccine was manufactured at Integrity Bio Inc. (Camarillo, California). The vaccine consists of ChulaCov19 mRNA encapsulated in a proprietary LNP delivery system developed by Genevant Sciences Corporation (Vancouver, British Columbia). ChulaCov19 was stored as a sterile suspension of 0.2 mg ml^{-1} at −75 ± 10 °C and diluted with normal saline according to the assigned dose, to be given at 0.5 ml intramuscularly (IM).

Trial procedures

Four adult sentinel participants were enroled to receive ChulaCov19 at 10 μg IM. Once no halting criteria (Supplementary Table 2) were reported by day 3, the remaining eight 10 μg participants were enroled. The same approach was followed for the 25- and 50 μg doses. The vaccine was administered in the deltoid muscle on day 1 and day 22 ± 3, followed by a 2 h safety monitoring on-site. Enrolment of the elderly participants was commenced after DSMB review of the data when the last adult participant of the 10 μg group had reached day 29 (1 week after the second vaccination) on study. A diary was provided to participants to record solicited and unsolicited adverse reactions, and concomitant medications for 7 d. Safety laboratory tests were performed at baseline and days 8, 22, 29 and 50.

Assessment of safety and tolerability

Safety endpoints included solicited and unsolicited local adverse events, systemic adverse events, use of antipyretics/analgesics in the 7 d after vaccination, and serious adverse events up to day 50 (4 weeks after the second vaccination). Castor EDC version 2021.2 was used for data collection.

Assessment of immunogenicity

Binding to the SARS-CoV-2 S1 RBD was measured by enzyme-linked immunosorbent assay (ELISA). Neutralizing antibody titres against WT and VOCs were assessed by MicroVNT-50 and PsVNT-50. SARS-Cov-2 RBD-ACE2 blocking antibody was measured by surrogate viral neutralization test. Cellular immunity was measured by IFNγ-ELISpot assay (ELISpot) and by intracellular cytokine staining (ICS) assay (Supplementary Table 3). Tests were performed on specimens collected at days 1, 8, 22, 29 and 50; however, PsVNT-50 was performed only at days 29 and 50. Exploratory comparator serum panels included (1) 30 HCS from adults with median age (s.d.) of 39.9 (16.6) years, 63.3% of whom were female and (2) 27 Pfizer/BioNTech mRNA vaccinees. Genotypic testing for the virus variant among human convalescents' sera was not available. However, based on the epidemiology data, it is likely to be wild-type SARS-Cov-2. The serum samples were collected at 4 weeks after confirmed diagnosis. All Pfizer/BioNTech mRNA vaccinees were Malaysian with median age (s.d.) of 34.5 (9.4) years, 77.8% of whom were female. Serum samples were collected at day 29 after the first dose (Supplementary Tables 4 and 5).

Assessment of vaccine stability

Samples of the ChulaCov19 vaccine cinical lots were stored at 3 different temperatures: −75 °C ± 10 °C, −20 °C ± 5 °C, 5 °C ± 3 °C. Samples were analysed at planned timepoints 1, 3, 6, 9, 12, 18 and 24 months; 3, 6 and 12 months; and 3 and 6 months, respectively, for each temperature.

The ongoing study is conducted at Intertek Pharmaceutical Services Laboratory in Manchester, UK (Supplementary Tables 13 and 14)

Statistical analysis

Sample size for phase I was based on practical and medical considerations rather than power for statistical hypothesis testing or precision of parameter estimation. Results of safety analyses are presented as counts and percentages, and group ages as mean (s.d.). Summary descriptive statistics relevant for study endpoints were provided for each cohort and vaccine dose group at the study timepoints indicated in the protocol.

Immunogenicity was analysed per protocol. GMTs and their 95% CIs were calculated for MicroVNT-50, PsVNT-50, anti-RBD-IgG, anti-S Trimer, sVNT, IFNγ-ELISpot and Th1/Th2 spike-specific CD4+ and CD8+ T cells. GMTs were calculated as exponentiated means of logarithmic-transformed assay results. Formal comparisons of MicroVNT-50, PsVNT-50, anti-RBD-IgG and sVNT against samples of 30 HCS from adults with COVID-19 and 27 Pfizer/BioNTech mRNA vaccinees were made using GMR (95% CI). The unadjusted and age-adjusted GMRs and 95% CIs were exponentiated with coefficients from the linear regression models, with natural log-transformed titres as outcome variables. Statistical analysis was conducted with Stata 17, and all statistical tests were two-sided.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data that support the findings of this study are available from the corresponding author upon request. Source data are provided with this paper.

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Author contributions

S.G., W.K. and K.R. managed the participants, developed the strategy plan, interpreted data, drafted and revised the manuscript. M.-G.A., W.W., A.C. and L.H. provided consultation for the investigational product and revised the manuscript. C.K., E.P., A.T., A.J., S.B., S.U. and T. Palaga performed the experiments and revised the manuscript. J.S., T.A. and S.K. performed the statistical analysis and revised the manuscript. A.K. assisted with the study design, provided reference samples and revised the manuscript. S.S., T. Puthanakit and K.P. managed the participants, developed the strategy plan and revised the manuscript. E.K. drafted and revised the manuscript. D.W. and K.R. conceptualized the work and strategy, provided consultation for the investigational product and revised the manuscript. K.R. conceptualized the work and strategy, supervised the study trial and revised the manuscript. All authors read and approved the final version of the manuscript for submission.

Competing interests

In accordance with the University of Pennsylvania policies and procedures and our ethical obligations as researchers, we report that Drew Weissman is named on patents that describe the use of nucleoside-modified mRNA as a platform to deliver therapeutic proteins and vaccines. We have disclosed those interests fully to the University of Pennsylvania, and we have in place an approved plan for managing any potential conflicts arising from licensing of our patents. The other authors declare no competing interests.

Additional information

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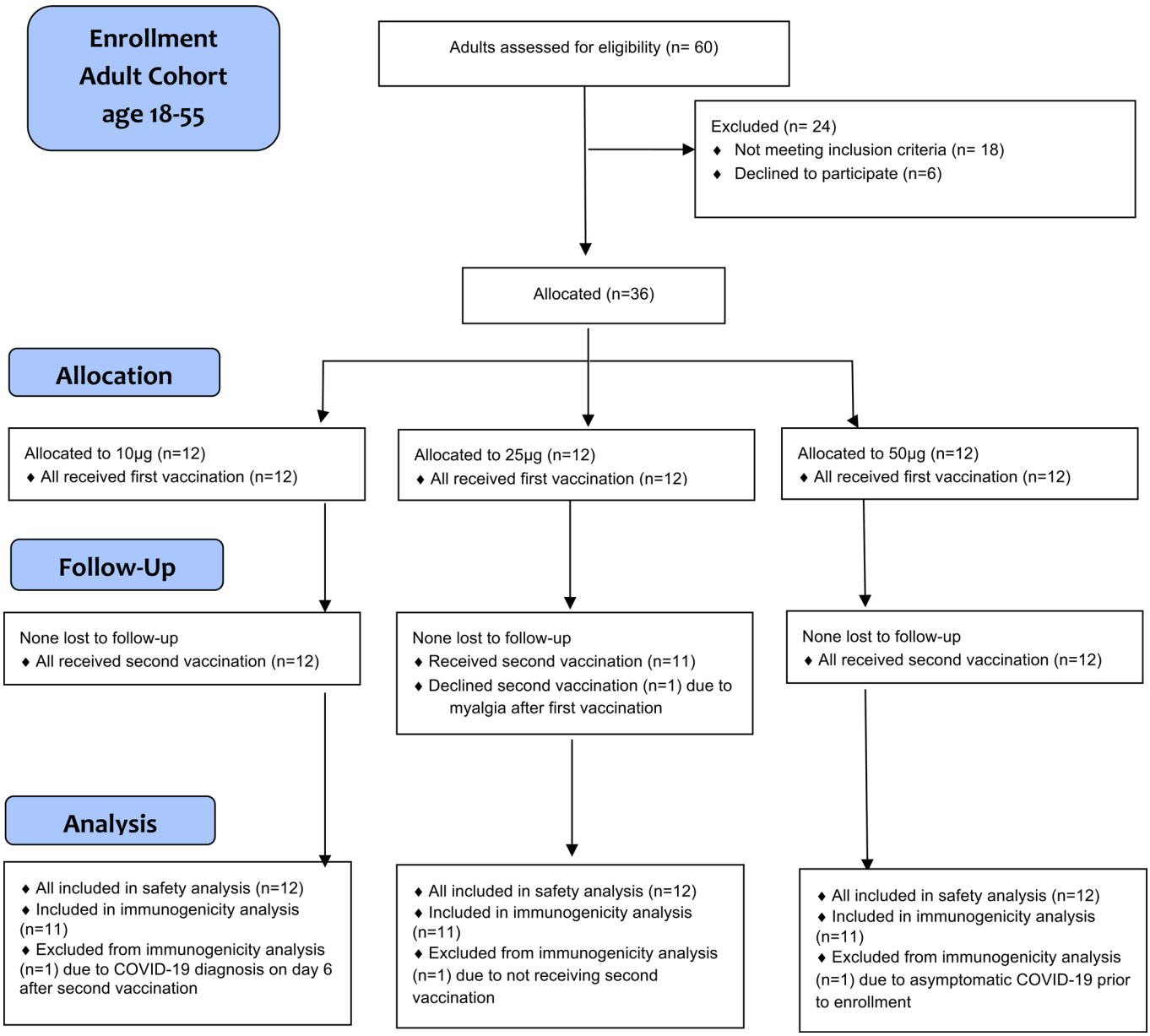

Extended Data Fig. 1 | Participants flow diagram of the adult cohort. All the participants received two doses of the ChulaCov19 vaccine, except for one participant who was assigned to receive 25 µg of ChulaCov19, who received one dose.

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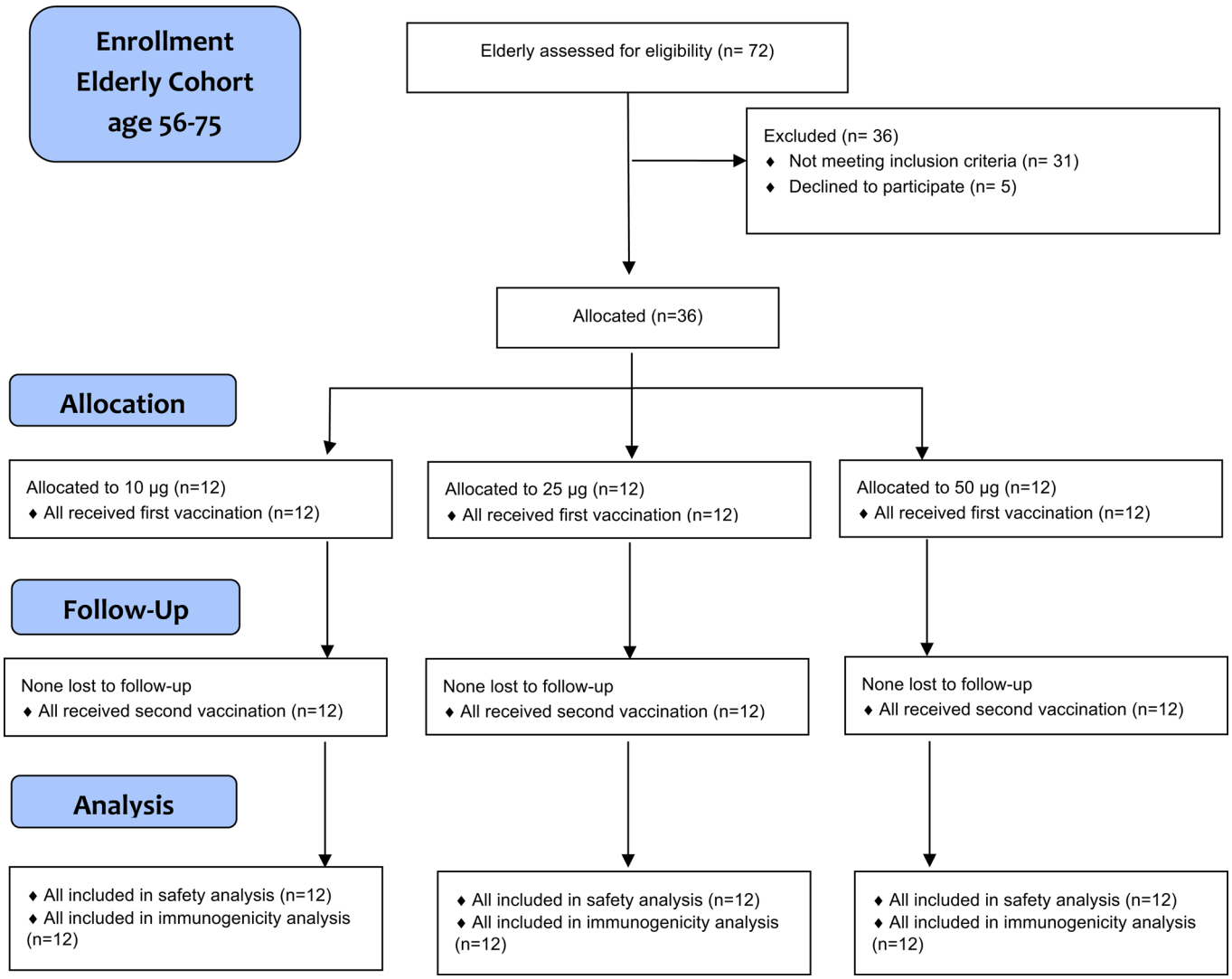

Extended Data Fig. 2 | Participants flow diagram of the elderly cohort. All the participants received two doses of the ChulaCov19 vaccine.

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☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	We used the Castor EDC version 2021.2 for data collection.
Data analysis	We used Stata version 17 for the data analysis.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

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- Accession codes, unique identifiers, or web links for publicly available datasets
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The data that support the findings of this study are available on request from the corresponding author (KR).

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	There was no restriction in enrolling the participants based on their gender. However, when we enrolled the participants, all of them were male and female.
Population characteristics	Mean age (SD) in the adult and elderly cohorts was 35.8(9.1) and 65.1(5.4) years, respectively; body mass index was 23(2.8) and 23.3(3.3); and proportion of females was 67% and 53%.
Recruitment	Study participants were recruited at the one site. We identified the interested individuals from the study website. Once recruited participants were screened for eligibility based on protocol. Eligible participants were then randomized to vaccine dose level in a blinded manner. These processes therefore did not led themselves to enrollment biases however participants who did not know about the study may have had less of an opportunity to participate.
Ethics oversight	The trial and the Investigational New Drug application were approved by the ethics committee of the Faculty of Medicine, Chulalongkorn University, Bangkok, and Thailand's Food and Drug Administration, respectively

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Since this is an early phase study, the sample size was determined on the basis of practical, logistical, and medical considerations and is not based on statistical power with regard to hypothesis testing, or precision with regard to parameter estimation. Accordingly, for this Phase 1/2 study, a total sample size of: up to 36 eligible healthy for each of the 2 age groups (the adult group: Group 1, and the elderly group: Group 2) volunteers. Sentinel dosing was performed in each dose-escalation cohort. The inclusion of 12 subjects per dose level is considered to be adequate for a safety assessment. The probability to observe a particular TEAE with incidence of 15% at least once in 12 subjects per group is 85.8%.
Data exclusions	We excluded 3 participants from the immunogenicity analysis. Because one participant refused to get the 2nd vaccination due to concern of side effect and the other 2 participants : one participant from the adult 10-µg group who was diagnosed with asymptomatic COVID-19 on the sixth day after the second vaccination and one participant from the adult 50-µg group who had confirmed positive baseline anti-RBD binding antibody test but was negative for SARS-CoV-2 molecular testing, indicating asymptomatic COVID-19 prior to enrollment.
Replication	This is an interim report of an ongoing phase 1/2 clinical trial. There was no attempt at replication of study findings. Participant sera and PBMCs were tested as single measurement.
Randomization	All participants meeting eligibility criteria were randomized sequentially in a 1:1:1 allocation ratio into each dose schedule.
Blinding	This was an open label study, no blinding.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used: The anti-human CD28/CD49d (BD Biosciences) were used with 0.1ug/ml dilution.

Validation: *Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.*

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration: NCT04566276

Study protocol: We have uploaded the study protocol to the Chula Vaccine Research Center (Chula VRC) website. https://www.chulavrc.org

Data collection: Data were collected at screening (up to 42 days before vaccination) and for randomized participants at the investigative site at baseline, 1 day, 7 days and 21 days, after Dose 1, 7 days after dose 2 and up to 28 days after dose 2.
Both safety and/or serum collection for immunogenicity assessments were collected for all stated time points. In addition, reactogenicity data were assessed through participant self reports via a paper diary for 7 days after each dose. There was also telephone follow-up at 24 and 48 hrs following dosing, to ensure subject well-being, which was documented on site by the study nurses conducting the call.

Outcomes: The following study primary outcomes are presented: the proportion of participants reporting prompted local reactions, systemic events, and use of antipyretic and/or pain medication within 7 days after vaccination, AEs and serious adverse events (SAEs) (available through up to ~50 days after Dose 1), and the proportion of participants with clinical laboratory abnormalities 7 days after vaccination and grading shifts in laboratory assessments between baseline and 1 and 7 days after Dose 1 and between Dose 2 and 7, 28 days after Dose 2.

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation: Fresh PBMCs were isolated from the peripheral blood by Ficoll-Hypaque density gradient centrifugation.
PBMCs were stimulated for 18h with SARS-CoV-2 spike peptides pools (2 µg/ml/peptide, Mimotope, Australia). Cells were stimulated with phorbol 12-myristate 13-acetate and ionomycin (Sigma) as a positive control. PBMCs were co-stimulated in the presence of 0.1 ug/ml anti-human CD28/CD49d (BD Biosciences) for 2 hours at 37oC with 5%CO2 and then incubated for an additional 16 hours after addition of brefeldin A and monensin to each well (BioLegend). Afterwards, cells were washed in FACS buffer (phosphate-buffered saline containing 2% fetal bovine serum; 2% FBS/PBS) and stained with a cocktail of surface antibodies (anti-human CD3-PerCP Cy5.5, CD4-APC-Cy7 and CD8- AF700; BioLegend) for 20 minutes at 4oC in the dark. After washed with FACS buffer, cells were fixed with 2% paraformaldehyde (PFA; Sigma, USA) and permeabilized with 1X BD Perm/ Wash buffer (BD Biosciences) for 15 minutes at RT in the dark. For intracellular staining, cells were stained with a cocktail of intracellular antibodies (anti-human IFN- -AF647, IL-2-PE and IL-4-PE/DZ594; BioLegend) for 30 minutes at RT in the dark. After washed with FACS buffer, cells were fixed with 2% PFA and analyzed by flow cytometry (LSRII, BD biosciences).
Fresh PBMCs were isolated from the peripheral blood by Ficoll-Hypaque density gradient centrifugation.

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Instrument: BD LSRII, BD biosciences

Software: FlowJo software V.10.8.1

Cell population abundance: fresh PBMC, no sorted cells

Gating strategy: Gating strategy of peripheral blood mononuclear cells (PBMCs) by flow cytometry. The lymphocytes were gated from FSC and SSC based on size and granularity. CD3 was used to identify T cells (CD3+) or non-T cells (CD3-). Staining with CD4 and CD8 antibody against CD4 and CD8 T cells from CD3+ T cells. Gated the IFN-gamma, IL-2 and IL-4 expression on CD4+ T cells or CD8+ T cells represented by percentage (%). SARS-CoV-2-specific cytokine production was corrected for background by subtraction of values obtained with medium. Negative control was set to zero. Cytokine production was calculated by the fractions of all CD4+ or CD8+ T cells positive for IFN- , IL-2 or IL-4. A positive response was defined as >0.2% after subtract background.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.